Effect of Selenium and Vitamin E on the Level of Sperm HSPA2+, Intracellular Superoxide Anion and Chromatin Integrity in Idiopathic Asthenoteratozoospermia: A Double-Blind, Randomized, Placebo- Controlled Trial

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Purpose: Male infertility accounts for about half of all infertility cases. Asthenoteratozoospermia is a severe form of male infertility. Free radicals play an important role in infertility. In a previous study we found that asthenoteratozoospermic men had a lower mean percentage of sperm HSPA2+ and higher intracellular anion superoxide than normozoospermia. Antioxidants are thought to be able to counteract the negative effects of free radicals. We explored the efficacy of vitamin E in combination with Se on the level of sperm HSPA2+, intracellular anion superoxide, and chromatin integrity in these patients.

Materials and methods: 60 patients entered the study. They were randomized to the treatment group of oral Se (200 µg) in combination with vitamin E (400 units) for 3 months (n = 30) or placebo (n = 30). Semen samples were obtained and assessed for sperm parameters, intracellular O2-, protamine deficiency, sperm HSPA2+ and apoptotic spermatozoa at baseline and after the treatment phase.

Results: There were no significant differences in baseline semen parameters, intracellular O2- protamine deficiency, sperm HSPA2+ and apoptotic spermatozoa between the treatment and placebo groups. There was a statistically significant decrease in sperm apoptosis and the level of anion superoxide (P = .001) and an increase in sperm motility and viability (P = .001) in the treated group, but no significant difference was found in the percentage of sperm HSPA2+ and sperm protamine deficiency compared with baseline. Moreover, no significant change was found in these parameters in the placebo group after 3 months.

Conclusion: Our results showed that administration of vitamin E and selenium for three months may improve sperm motility and viability by decreasing intracellular anion superoxide and sperm apoptosis in asthenoteratozoospermic infertile men. We suggest that consuming these supplements before assisted reproductive technology (ART) may improve outcomes in these patients.

Keywords: infertility; male; selenium; vitamin E; HSPA2 protein

INTRODUCTION

Male factor infertility is a common cause of infertility and accounts for about 30% to 40% of infertility. Common causes of male infertility include gene mutations, aneuploidy, varicocele, radiation, chemotherapy, genital tract infections, and erectile dysfunction[1].

But sometimes other factors such as high levels of reactive oxygen species (ROS) can affect sperm as they pass through the ducts, causing subfertility or infertility. It has been reported that ROS may be a causative factor in 30–80% of infertile men[2].

The cause of infertility in about 60%–75% of infertile men is unclear. This is called idiopathic infertility[3].

Sperm are able to produce low levels of ROS, which are involved in their critical functions.

As a result of an error in spermiogenesis, sperm with a large amount of extra cytoplasm are released. This extra cytoplasm contains additional enzymes that promote ROS production by the redox system in the cytoplasmic membrane. Superoxide anion and hydroxyl radicals are the most important free radicals from oxygen derivatives. Oxidative stress (OS) occurs as a result of an imbalance between the produced ROS and antioxidant defense, neutralizing their toxicity and resulting in sperm DNA damage, reduced sperm motility, and fertilization potential[4,5].

On the other hand, a study reported that the percentage of sperm HSPA2+ in infertile individuals was lower than in fertile individuals. HSPA2 plays a role in repairing DNA fractures and replacing histones with protamine during nuclear sperm compaction. Decreased expression of this protein is associated with increased aneuploidy, DNA fragmentation, apoptosis, and defect in histone - protamine translocation[6-8].

In a previous study in this field, it was also found that infertile patients with asthenoteratozoospermia had a lower mean percentage of sperm HSPA2+ and higher intracellular anion superoxide than normozoospermic men[9].

One of the most important antioxidant molecules against oxidative damage is vitamin E. This molecule is mainly located in the cell membrane and it prevents li-

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Table 1. Demographic characteristics, sperm analysis and the DHE CMA3, HSPA2 and YO levels before treatment in two study groups

Sperm Parameters	Vit E+ selenium(n=30)	Placebo(n=30)	*P*- value
Male age, year; mean ± SD (range)	31.90 ± 3.68	33.46 ± 3.72	0.107
Sperm volume. ml; mean ± SD (range)	3.35 ± 0.35	3.51 ± 0.27	0.125
Sperm PH; mean ± SD (range)	7.31 ± 0.13	7.27 ± 0.07	0.467
Vitality; mean ± SD (range)	55.16 ± 14.84	56.64 ± 14.24	.975
Sperm concentration ×106/ml; mean ± SD (range)	40.46 ± 18.51	43.86 ± 19.66	.893
Total motility, %; mean ± SD (range)	28.88 ± 6.66	29.47 ± 5.77	.987
Normal morphology, %; mean ± SD (range)	1.83 ± .74	2.03 ± .80	.799
CMA3+,%; mean ± SD (range)	37.06 ± 6.24	35.69 ± 5.05	.782
HSPA2+ ,%; mean ± SD (range)	22.26 ± 7.48	22.56 ± 6.37	.998
DHE+ ,%; mean ± SD (range)	35.40 ± 6.26	35.02 ± 5.58	.995
YO+ ,%; mean ± SD (range)	37.07 ± 8.40	40.97 ± 8.75	.345

Abbreviations: CMA3= Chromomycine A3 Staining, HSPA2= Heat-shock protein A2, DHE = Dihydroethidium, YO= Yo-pro-1 Iodide.
Note: Values are presented by mean± SD. Paired sample t-test was used to compare dependent variables. P-value<0.05 was considered statistically significant.
By comparing the hormonal levels in Sertraline and control group we founded that FSH, LH and testosterone levels all increased in the Sertraline group, but this increase was only significant for FSH ($P < 0.05$). There was no significant difference in LH, FSH and testosterone levels between the 80th day and 170th day in the control group ($P > 0.05$)

pid peroxidation and cell membrane damage by neutralizing free radicals and enhancing other antioxidants[10]. Selenium (Se) is an essential dietary micronutrient required for reproductive functions such as testosterone metabolism and about 20-40% of infertile men who have deficiency in sperm production have been linked to selenium deficiency[11]. Kaushal et al. found that variation in the amount of selenium can lead to OS and thereby affect reproductive potential[12]. This demonstrates the importance of nutrition at the molecular level.
In this study, we investigated the effect of daily oral supplementation of Se and vitamin E on the level of intracellular superoxide anion, sperm HSPA2 +, protamine deficiency, and sperm parameters in teratoasthenozoospermia men.

MATERIALS AND METHODS

A double-blind, randomized, placebo-controlled trial study was conducted on 60 infertile men with asthenoteratozoospermia (ATZ) (with normal morphology lower than 4% and total motility lower than 40%) at Royan Research and Clinical Center for Infertility (Tehran, Iran) from June 2014 to June 2016.
All participants in this study were blinded to the intervention until the study was completed.
Each participant randomly received two packs of pills in different colors from a doctor who was unaware of their contents and the record book remained in their hands until the end of the study. The treatment group received a daily supplement of vitamin E (400 IU) combined with selenium (200µg)[13] and the placebo group received two tablets of placebo for 3 months.
Also, all patients were followed up by researchers during the study, and any adverse events were addressed.
Out of 117 patients were included in this study, 54 patients were excluded due to drug cessation and incomplete consumption (n = 12) and/or declined to participate (n = 42) (**Figure 1**).
Semen samples were evaluated in two groups, treatment and placebo. Participants who met the study criteria, and who consented to participate were entered into the study. Inclusion criteria comprised history of infertility of at least one year despite regular unprotected intercourse; seminal analysis showing normal morphology lower than 4% and total motility lower than 40% as defined by WHO manual for semen analysis, (2010). Exclusion criteria comprised the cases with leukocytospermia (>1×10^6 WBC/mL), oligo and azoospermia, varicocele, cancer, endocrine disorders, genital tract infection, autoimmune disease, cryptorchidism, smoking or alcohol consumption which may impact the intracellular ROS, and patients who received chemotherapy, radiotherapy, and recent antioxidant intake. The study adhered to the local ethical protocol. Semen samples were obtained at baseline and after the treatment phase and were analyzed in accordance with WHO criteria.

Semen collection and analyses

Semen samples were obtained by masturbation after sexual abstinence of 2 to 4 days. After liquefaction in the lab at 37°C , samples were assessed for sperm parameters, intracellular O2, protamine deficiency, sperm HSPA2+, and apoptotic spermatozoa.

Semen Analysis

Semen samples were collected into sterile containers in the laboratory by masturbation and after complete liquefaction at room temperature (22º C) for 30 min, they were assessed for macroscopic parameters such as color, pH, ejaculate volume, and viscosity. An aliquot of the sample was evaluated for sperm concentration, total motility, and morphology according to WHO criteria (WHO, 2010)[14].
First, sperm concentration and total motility were assessed by CASA, then reanalyzed manually by a single experienced technician. The semen samples were mixed well; 10 µl of the sample was placed on a clean glass slide that had been stored at 37 °C and it was covered with a coverslip.
The samples were placed on the heating stage of a microscope at 37 °C and were immediately observed at ×400 magnification.
Sperm vitality and morphology in 200 spermatozoa per slide were evaluated by two experienced technicians using the Eosin/Nigrosine and Papanicolaou staining, respectively. The same experienced technician performed all the semen analyses.

Assessment of intracellular O2, HSPA2 and apoptotic spermatozoa by flowcytometry

DHE (Dihydroethidium) is a specific probe for O2-. and a cell permeable stain. Sperm suspension was incubated with DHE (1.25µM; Sigma) at 25 °C for 25

Table 2. Comparison of the levels of LH, FSH and Testosterone between 80th and 170th day in Sertraline and control group.

Sperm Parameters	Vit E+ selenium(n=30)	Placebo (n=30)	*P*- value
Sperm volume, ml; mean ± SD (range)	3.06 ± 0.21	3.32 ± 0.19	0.001
Sperm PH; mean ± SD (range)	7.25 ± 0.11	7.33 ± 0.08	0.02
Vitality; mean ± SD (range)	69.86 ± 12.56	46.75 ± 12.74	0.0001
Sperm concentration ×106/ml; mean ± SD (range)	40.66 ± 17.15	42.20 ± 18.93	.989
Total motility, %; mean ± SD (range)	44.39 ± 8.91	29.11 ± 5.09	0.0001
Normal morphology, %; mean ± SD (range)	2.16 ± 1.05	1.66 ± 0.75	.110
CMA3+,%; mean ± SD (range)	36.76 ± 5.96	37.13 ± 5.11	.994
HSPA2+,%; mean ± SD (range)	22.49 ± 7.10	22.08 ± 5.58	.995
DHE+,%; mean ± SD (range)	26.61 ± 7.66	38.96 ± 5.13	0.0001
YO+,%; mean ± SD (range)	28.34 ± 10.58	44.02 ± 8.36	0.0001

Abbreviations: CMA3= Chromomycine A3 Staining, HSPA2= Heat-shock protein A2, DHE = Dihydroethidium, YO= Yo-pro-1 Iodide
Note: Values are presented by mean ± SD. Independent sample t-test was used to compare dependent variables. *P*-value < 0.05 was considered statistically significant.

min. DHE is oxidized by O2- and produces ethidium bromide which binds to the sperm DNA and emits red fluorescence which is then analyzed by a flowcytometer (FACS Caliber; BD Biosciences, USA) between 590 and 700 nm. Yo-pro-1 Iodide (Y3603- Life Technology) was used as a counterstain dye for DHE and excluded the apoptotic spermatozoa[9,15].

To measure the percentage of HSPA2+ spermatozoa, all samples were washed twice in cold phosphate – buffered saline (PBS, Gibco, USA), 4% paraformaldehyde was added and samples were incubated for 20 min at room temperature, and then centrifuged for 5 min at 300g. Test fractures were permeable in 5% Triton X-100 for 5 min and they were incubated overnight with the primary anti-HSPA2 antibody (Santa Cruz Co.) at a dilution of 1:100 in 3% bovine serum albumin (BSA; Sigma Co.) at 4 °C; Control samples were incubated under the same conditions with 3% BSA. Two samples were washed and incubated with PE-conjugated Donkey anti-goat IgG (1:200, Santa Cruz Co.) in 1.5% BSA at 4 ^{0}C for 1 h. After washing, BD FACS Caliber flow-cytometry was used for further analysis[16]. We assessed at least 10000 spermatozoa in each sample using the flowcytometry software (Flowjo 7.6.1) and expressed in percentage.

Chromomycin A3 (CMA3) staining for Protamine deficiency assessment

Chromomycin A3 (CMA3), is an indirect assessment for protamine content that competes with protamine to bind DNA. Using this procedure the semen sam-

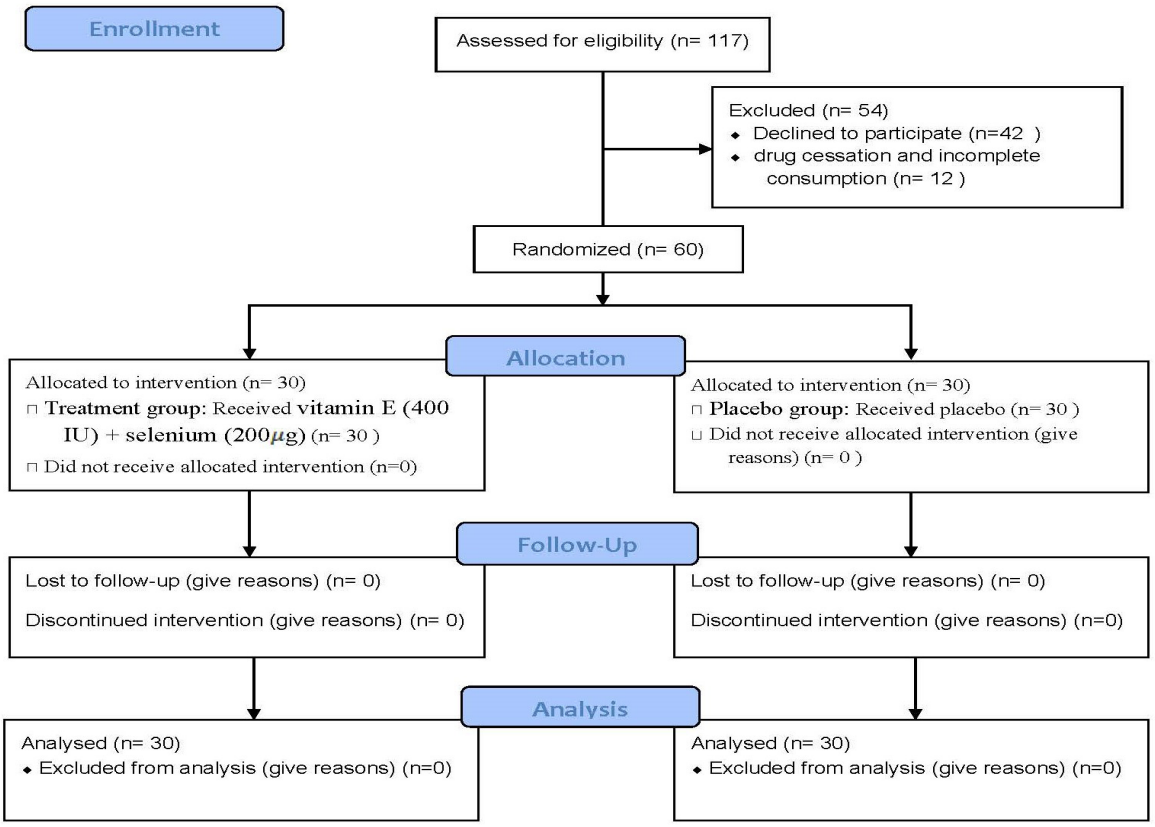

Figure 1. Study design, and distribution of patients into treatment

Table 3: Comparison of the pre and post treatment seminal parameters, the level of DHE CMA3, HSPA2 and YO in two study groups.

Sperm Parameters	Vit E+ selenium(n=30) Pre-treatment	Post-treatment	P- value	Placebo (n=30) Pre-treatment	Post-treatment	*P*- value
Sperm volume. ml; mean ± SD (range)	3.35 ± 0.35	3.06 ± 0.21	0.0001	3.51 ± 0.27	3.32 ± 0.19	0.045
Sperm PH; mean ± SD (range)	7.31 ± 0.13	7.25 ± 0.11	0.171	7.27 ± 0.07	7.33 ± 0.08	0.102
Vitality; mean ± SD (range)	55.16 ± 14.84	69.86 ± 12.56	.0001	56.64 ± 14.24	46.75 ± 12.74	.030
Sperm concentration ×106/ml; mean ± SD (range)	40.46 ± 18.51	40.66 ± 17.15	1.000	43.86 ± 19.66	42.20 ± 18.93	0.986
Total motility, %; mean ± SD (range)	28.88 ± 6.66	44.39 ± 8.91	0.0001	29.47 ± 5.77	29.11 ± 5.0900	.997
Normal morphology, %; mean ± SD (range)	1.83 ± .746	2.16 ± 1.05	.430	2.03 ± .80	1.66 ± .75	0.345
CMA3+, %; mean ± SD (range)	37.06 ± 6.24	36.76 ± 5.96	.997	35.69 ± 5.05	37.13 ± 5.14	0.756
HSPA2+, %; mean ± SD (range)	22.26 ± 7.48	22.49 ± 7.10	.999	22.56 ± 6.37	22.08 ± 5.58	0.992
DHE+ , %; mean ± SD (range)	35.40 ± 6.26	26.61 ± 7.66	.0001	35.02 ± 5.58	38.96 ± 5.13	0.074
YO+ , %; mean ± SD (range)	37.07 ± 8.40	28.34 ± 10.58	.002	40.97 ± 8.75	44.02 ± 8.36	0.563

Abbreviations: CMA3= Chromomycine A3 Staining, HSPA2= Heat-shock protein A2, DHE = Dihydroethidium, YO= Yo-pro-1 Iodide
Note: Values are presented by mean± SD. Paired sample t-test was used to compare dependent variables. P-value<0.05 was considered statistically significant.

ples were washed with PBS, smears of them were prepared and dried. Then, they were fixed in Carnoy,s solution (Methanol/Glacial acetic: 3:1) at 4 ^{0}C for 10 min, stained with 100µl of CMA3 (0.25 mg/mL) (Sigma Co.) for 25 min in dark at room temperature and mounted with buffered glycerol. We counted 200 spermatozoa under a fluorescence microscope at 1000x magnification in all samples. Spermatozoa with normal protamine content (CMA3 – negative) and spermatozoa with protamine deficiency are stained dull green and bright yellow, respectively[9].

This study was approved by Institutional Review Board of Yazd Research and Clinical Center for Infertility and informed consent forms were signed by all participants. Ethical committee registration: Yazd Research and Clinical Center for Infertility, Ethical code: 342/26/693 Clinical trial registration: IRCT20140409017210N2

Statistical analysis

Statistical analysis was performed using SPSS software, (version 16.0, SPSS Inc., Chicago, IL, USA). The data distribution was normalized with K-S test. Independent sample t-test was used to compare two study groups. Paired sample T test was used to compare pre and post-treatment parameters. One-way analysis of variance (ANOVA) was used for comparison of parameters between groups. Two tailed p-value less than 0.05 was considered as statistically significant outcome for the measured cases. All data was presented as mean ± standard deviation.

RESULTS

Demographic characteristics in two study groups before treatment (Vit E+ Selenium and Placebo) are summarized **Table 1**. Demographic characteristics and sperm parameters were similar between the two groups. Also, comparison of post-test seminal parameters, the level of DHE, CMA3+, HSPA2+ and YO+ between two groups are shown in **Table 2**. Comparison of pre and post treatment seminal parameters, the level of DHE, CMA3+, HSPA2+ and YO+ in two study groups are summarized in **Table 3**.

Sperm concentration

There were no differences between the two groups before treatment (p = .893) and post treatment (p = .989) (**Tables 1 and 2 respectively**). There were no statically significant changes in sperm concentration in placebo (p = .986) and Vit E+ selenium group (p = 1.000) groups after 3 months (**Table 3**).

Sperm total motility

There was no difference between two groups before treatment (p = .987), but it was significant post treatment (p = .0001) (**Table 1 and Table 2**).While for the placebo group no change was observed (p = .99), the increase in the Vit E+ selenium group was highly significant (p = .0001) (**Table 3**).

Normal morphology

There were also no differences between the two groups before treatment (p = .799) and post treatment (p = .110). In placebo (p =.345) and treatment group (p =. 43, no significant changes were observed after 3 months.

Sperm vitality

There was no difference for the comparison between two groups at the baseline (p = .975) but it increased in the treatment group significantly. (p = .0001) (**Table 1 and Table 2, respectively**). While sperm vitality decreased in the placebo group significantly after 3 months (p =.030), and it increased in Vit E+ selenium group significantly (p = .001) (**Table 3**).

Semen volume

There was no statistically significant difference between the two groups before treatment (**Table 1**). Comparison between post-treatment groups showed changes were significant and in the placebo group volume increased significantly (**Table 2**). Pre and post-treatment comparison showed a significant decrease in volume in VIT E+ selenium group (**Table 3**).

Sperm pH

There was no statistically significant difference between the two groups before treatment (**Table 1**). Comparison between post-treatment groups showed changes were significant and in the placebo group pH increased significantly (**Table 2**). Pre and post-treatment comparison showed there was no statistically significant difference between pre and post-treatment with Vit E+ selenium (**Table 3**).

Percentage of sperm CMA3+

There was significant difference between the two groups pre-treatment (p = .782) and post-treatment (p = .994) (**Table 1 and Table 2**). No changes occurred in percentage of sperm CMA3+ in placebo Vit E+ selenium group, (p =.756) (p =.997), after 3 months (**Table 3**).

Percentage of sperm HSPA2+

There were also no differences between the two groups pre-treatment (p = .998) and post-treatment (p = .995) (Table 1 and Table 2). Percentage of sperm HSPA2+ +,

in both groups, showed no significant changes after 3 months (**Table 3**).

Intracellular O2-

There was no difference between the two groups before treatment (p = .995) but it was decreased in post-treatment significantly (p = .0001) (**Table 1 and Table 2**). Pre and post-treatment placebo groups showed no change (p = .074) in the level of intracellular O2- , though in the VitE+ selenium group it decreased significantly after 3 months(p =.0001) (**Table 3**).

Percentage of apoptotic spermatozoa

There was no difference between the two groups before treatment (p = .345) but a significant decrease was observed in VitE+ selenium group (p = .0001) (Table 1 and Table 2). In the placebo group, no change was observed (p = .563), but in the Vit E+ selenium group, the percentage of apoptotic spermatozoa decreased significantly after 3 months (p =.002) (Table 3).

DISCUSSION

Infertility is a common medical and social problem that affects about one out of eight couples and approximately 40–50% is due to "male factor" [16, 17]. Asthenoteratozoospermia is a severe form of male infertility and our findings in the previous study showed that they have a higher level of intracellular superoxide anion compared to normosospermic men[9].

Antioxidants are molecules that are able to reduce or inhibit oxidative stress by scavenging free radicals. When the concentration of free radicals in the body increases, the endogenous antioxidant system is compromised and unable to fully protect the body. In this situation, the use of exogenous antioxidants in dietary supplements or medications can be helpful[18].

There are several antioxidants in seminal plasma which improve sperm quality. Some of them are vitamins E and C, along with selenium and zinc, which are constituents of the antioxidant system[19].

Selenium is an essential element in the biosynthesis of the hormone testosterone and sperm formation and is the constituent of different selenoproteins. At least 25 selenoproteins in human and animals are known and are involved in maintaining the normal structure of sperm [20]. 20 to 40% of infertile men whose infertility is related to decreased sperm production is due to selenium deficiency[21]. It is involved in the structure of the enzyme glutathione peroxidase, which is an important antioxidant and a marker for oxidative stress[22].

Oral supplementation of selenium (50 microgram) has been reported to significantly increase in sperm parameters, serum testosterone and glutathione levels. Also, serum MDA significantly decreased in patients after treatment[11]. In a study, selenium levels were assessed in idiopathic infertile men. In this study, selenium concentration in the seminal plasm and sera of 60 infertile men with oligospermia and azoospermia was measured (case group) along with 40 fertile men with normozoospermia (control group). They concluded that the mean serum selenium level in infertile men with oligospermia was significantly higher than in infertile men with azoospermia and a significant inverse relationship between selenium levels and sperm count was found. They also found that there was a relationship between selenium levels in the plasma seminal and other sperm parameters[23].

Hamza et al. investigated the protective and antioxidant effects of selenium nanoparticles (Se NP) on testicular structure changes in male mice treated with monosodium glutamate (MSG). SeNP is known as a flavor enhancer that has toxic effects on the male reproductive system. They found that, SeNP inhibit testicular injury and improve the antioxidant state in male mice treated with MSG[24]. In a study by Scott et al., 96 infertile men were treated with selenium or selenium in combination with vitamin E, A and C and they showed significant improvements in sperm motility[25].

In another study, selenium and N-acetyl-cysteine administration in infertile men with idiopathic oligo-asthenoteratospermia for 30 weeks improved all sperm parameters[26].

Vitamin E is a fat-soluble vitamin that is able to neutralize free radicals and protects cell membranes against the ROS by preventing lipid peroxidation as well as enhancing the function of other antioxidants[27]. In summary, the mechanism by which vitamin E protects cells from oxidative stress includes maintaining normal glutathione levels as an intracellular scavenger of free radicals, protecting cell membranes by inhibiting peroxidation, and clearing cells of ROS[28,29], and reduced apoptosis[30].

On the effect of vitamin E, Keshtgar et al. showed one hour invitro incubation of semen samples from teratozoospermia patients with vitamin E significantly increased sperm motility and viability, but sperm DNA fragmentation and acrosome reaction did not change [31]. Kemal Ener et al. found that oral administration of vitamin E increased sperm parameters after varicocelectomy but it was not statistically significant[32]. Inhibition of ROS production in infertile men with vitamin E administration has also been reported[33]. Abad and colleagues designed a study in which 20 infertile asthenoteratozoospermic men were treated with a multi antioxidants combined with vitamin E and selenium for 3 months. The results showed a significant improvement of DNA integrity and significant increase in concentration, motility, vitality, and normal morphology[34].

In 2020, Matorras R. et al. examined the effect of vitamin E administration on men from infertile couples on sperm parameters and ART results in a double-blind randomized study. Vitamin E improved sperm parameters but they did not find a significant difference between the vitamin E treatment and the placebo groups. However, administration of vitamin E significantly increased live birth rates compared with placebo, and a tendency to achieve better results was seen in other IVF parameters in the treatment group[35].

In this study, the effect of Se as a component of antioxidant system and Vitamin E on different parameters, intracellular anion superoxide, sperm apoptosis, and sperm chromatin deficiency was studied. Abad and colleagues designed a study in which 20 infertile asthenoteratozoospermic men were treated with multi antioxidants combined with vitamin E and selenium for 3 months. The results showed a significant improvement of DNA integrity and a significant increase in concentration, motility, vitality, and normal morphology[34].

Moreover, Moslemi et al. confirmed the protective and beneficial effects of Selenium–vitamin E supplementation on semen parameters and pregnancy rate. In this study, idiopathic asthenoteratospermia men received daily supplements of vitamin E in combination with selenium for 100 days and they showed an increase in

sperm motility and morphology[13].

Our investigation showed that Se (200 µg) in combination with vitamin E (400 units) for 3 months has a potential effect on the reduction of sperm apoptosis and level of anion superoxide and also increases sperm motility and viability (**Table 3**). In addition, we found that in comparison with posttest in the treatment group, the level of intracellular O2.- and apoptotic spermatozoa were lower than placebo group significantly in the final visit (p = .0001) (**Table 2**); also, sperm total motility and vitality were significantly higher (p = .0001) (**Table 2**).

High levels of intracellular superoxide anion in men with asthenoteratozoospermia[9] can increase sperm apoptosis. On the other hand, the high presence of polyunsaturated phospholipids in sperm membrane, makes it very sensitive to high levels of ROS and increases the processes of lipid peroxidation[36], reducing sperm motility and vitality.

Vitamin E is able to directly neutralize the anion superoxide[37]. In addition, a significant positive correlation has been found between serum selenium concentration and glutathione peroxidase (GSH-PX)[38]. GSH is a major antioxidant enzyme and a selenoprotein which protects the organism from oxidative stress by reducing reactive oxygen species[39].

Therefore, according to the results of the current study, we may be able to say that, vitamin E and selenium in these patients play a role in free radicals removal and reduction of ROS and this reduction can lead to improving sperm motility, viability, and apoptosis.

Also, we observed no change in the level of sperm protamine deficiency and HSPA2 after treatment. HSPA2 is involved in histone-protamine translocation. Therefore, no change in sperm protein deficiency after treatment may be attributed to the lack of change in HSPA2 level.

According to the inclusion and exclusion criteria, the selection of patients was one of the major limitations of this study. Because, many patients with asthenoteratozoospermia consumed alcohol or drugs in varying amounts, and some took a variety of antioxidants and medications, and this makes it very difficult to follow these patients.

Another problem was the measurement of intracellular superoxide anion by flow cytometry in this trial study. Because it is a compound that must be quickly coordinated and measured with the flow cytometry department.

Based on the results of this study, we propose other studies in higher populations in which pregnancy rates are also measured.

CONCLUSIONS

Our results showed that the administration of vitamin E and Selenium for three months decreased the level of intracellular anion superoxide in asthenoteratozoospermic men which may lead to improved motility, reduced apoptosis, and increased sperm viability in these patients. However, it does not affect the sperm HSPA2 + as well as sperm protamine deficiency level in them. In conclusion, administration of vitamin E and selenium may have a positive effect as a low-cost supplement to improve sperm parameters in infertile asthenoteratozoospermic men and prescribing them before using assisted reproductive techniques (ART) may improve outcomes. We also believe that the limitation of changes in sperm parameters to the period of administration may be due to the lack of effect of these supplements on factors such as HSPA2 in these patients.

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