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Effects of Different Antioxidants on Sperm Parameters in Infertile Males

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Abstract
Infertility is one of the most serious social problems facing all communities. The aim of this randomized controlled study is to assess the effect of certain antioxidants on the semen parameters. 105 infertile males aged between 20 and 40 years with oligoasthenoteratospermia from the Infertility Clinic in Shatby University Hospital were subjected to routine investigations, Computer Assisted Semen Analysis (CASA) and scrotal ultrasonography. They were allocated to 3 groups (35 males each); first group received vitamin C 1000 mg and zinc 20 mg, second group received Acetyl cysteine 200 mg and selenium 100 µg, and third group received vitamin E 1000 mg and folic acid 400 µg, once daily for 12 weeks. Sperm concentration among the three studied groups before and after treatment was not statistically significantly different (p=0.682, 0.500; respectively). Sperm motility increased after treatment in the three studied groups (p=0.000). Normal forms of sperms increased after treatment when compared with before treatment in the three studied groups (p=0.000). Combination of oral antioxidant therapy is important for the improvement of one or more of sperm parameters in infertile male with oligoasthenoteratozoospermia. This study suggests that N-Acetyle-Cysteine and selenium are the appropriate antioxidant combination but future studies should be done on wider scale of antioxidants to choose the best to serve humanity.

Introduction

Infertility is defined as inability of a couple to conceive after 12 months of regular unprotected sexual intercourse.[1]

Male factor infertility constitutes about half of all cases of infertility.[2] There are many causes of male infertility which may be caused by smoking, urinary tract infection, exposure to radiation, genital tract infection and oxidative stress.[3]

Oxidative stress is one of the most important factors contributing to poor quality of semen which occurs when the production of Reactive Oxygen Species (ROS) exceeds the natural antioxidant defense in the body.[4]

Leucocytes (particularly neutrophils and macrophages) and Immature spermatozoa are the two main sources of ROS.[5]

ROS generated from Spermatozoa play an important role in the process of sperm capacitation, so maintenance of suitable level of ROS is essential for adequate sperm function while excessive ROS impairs sperm motility and alter sperm morphology.[6]

Spermatozoa are protected by different forms of antioxidants and antioxidant enzymes found in the seminal plasma or in the spermatozoa itself to prevent oxidative damage and DNA fragmentation of sperm DNA.[7]

Vitamin C, vitamin E, glutathione, thioredoxin and superoxide dismutase are common antioxidants in semen which neutralize free radical activity and save sperms from the harmful effects of ROS that is already produced.[8]

Ascorbic acid also known as vitamin C is a potent water soluble antioxidant acting as a scavenger for a wide range of ROS. It is present approximately tenfold in a higher concentration in the seminal plasma than in blood and mostly secreted from seminal vesicle.[9]

Vitamin E is a fat soluble antioxidant. The best established biochemical function of vitamin E is its action as a lipid antioxidant. Also it inhibits production of ROS and protects cellular membrane against O_2 free radical.[10]

Folic acid is one of vitamin B, it is essential for synthesis of RNA, DNA and metabolise amino acids which are required for cell division. DNA synthesis plays an important role in the germ cell development and therefore it is obvious that folate is important for reproduction.[11]

Selenium is an essential trace element which is important for reproductive functions such as testosterone metabolism and is a constituent of sperm capsule.[12] The antioxidant effect of Selenium is important for testicular development, spermatogenesis and spermatozoa motility and function.[13]

Zinc is the second most abundant metal in the body after iron. It is present in high concentration in the seminal fluid. It affects the stability of sperm chromatin and protects spermatozoa against bacteria.[14]

N-acetyl-cysteine. It is an amino acid that has antioxidant properties and reduces the oxidative stress by scavenging free radicals. It is converted in the body to cysteine, which is a precursor to the biological antioxidant glutathione.[15]

Subjects and Methods

In this randomized controlled study, one hundred and five infertile males aged 20 to 40 years with oligoasthenoteratospermia were selected from the infertility clinic in Shat by Hospital and included in the study from February 2016 till January 2017.

Inclusion criteria
- Male factor primary infertility

Exclusion criteria
- Azospermia
- Testicular atrophy
- Hepatitis C
- Drug addicts

Method of randomization

The allocation sequence was generated using permuted block randomization technique and the block size was variable.[16] Allocation sequence/code was concealed from the person allocating the participants to the intervention arms using sealed opaque envelopes.[17] Double blinded approach was adopted. Masking/blinding was employed to outcome assessors and statisticians who were blinded to group allocation of patients.

All cases were subjected to
1. Full history with special attention to special habits, drug history, medical history and operative history.
2. Investigations including:
   a. Routine investigations (complete blood count, fasting blood sugar, liver and renal functions tests).
   b. Computer Assisted Semen Analysis (CASA).
   c. Scrotal ultrasonography.

The selected cases were allocated to 3 groups:
a. Group A: 35 cases who received vitamin C 1000 mg tablets and zinc 20 mg tablets once daily orally for 12 weeks.
b. Group B: 35 cases who received Acetyl cysteine 200 mg effervescent in 100 ml water and selenium 100 mcg tablets once daily orally for 12 weeks.

c. Group C: 35 cases who received vitamin E 1000 mg tablets and folic acid 400 mcg capsules once daily orally for 12 weeks.

Statistical methodology

- Data were collected and entered to the computer using SPSS (Statistical Package for Social Science) program for statistical analysis (ver 21).[18] Data were entered as numerical or categorical, as appropriate.
- Kolmogorov-Smirnov test of normality revealed significance in the distribution of the variables, so the non-parametric statistics was adopted.[19]
- Data were described using minimum, maximum, median and inter-quartile range.

- Categorical variables were described using frequency and percentage of column.
- Comparisons were carried out between more than two studied independent not-normally distributed subgroups using Kruskal-Wallis test.[20]
- Post-hoc pair-wise comparisons when Kruskal-Wallis test was significant were carried out using Dunn- Sidak test for multiple comparison.[21]
- Comparisons were carried out between two studied related not-normally distributed subgroups using Wilcoxon Signed Ranks test.[22]

Percentage change was calculated as follows:

$$\textbf{Percentage change}\ (\%) = \frac{\text{Measurement (after)} - \text{Meaurement (before)}}{\text{Meaurement (before)}}\ \text{x } 100$$

- Chi-square test was used to test association between qualitative variables. and Monte Carlo correction[23] was carried out when indicated (n x m table.

An alpha level was set to 5% with a significance level of 95%, and a beta error accepted up to 20% with a power of study of 80%.

Results

In the present study, age in group A (vitamin C and zinc) ranged from 20 to 40 years, with a median and Inter-quartile range (IQR) of 28 (25.00-31.00) years, while age ranged from 21.00-39.00 years in group B (Acetyle cysteine and selenium) with a median (IQR) of 32.00 (27.00-34.00) years, in group C (Vitamin E and folic acid) it ranged from 21.00-39.00 years with a median (IQR) of 28.00 (23.00-33.00). There was a statistical significant difference in age among the three studied group (X^2=6.236, p=0.044), but this statistical difference could approach statistical significance difference when pair-wise comparison was carried out (Figure 1). Most of patients included in this study were smokers, 31

(88.57%) in group A, 31 (88.57%) in group A, 29 (82.86%) in group B and 29 (82.86%) in group C. Prevalence of smoking was not statistically different among the three studied groups (X^2=0.590, p=0.832). Operative history of varicocele and medical history of diabetes mellitus were statistically not significant among the three studied groups (p=0.167, 0.907; respectively) (Table I).

Sperm concentration before treatment was statistically not significant among the three studied groups (p=0.682), and after treatment (p=0.500). Also percentage change of sperm concentration after treatment was statistically not significant among the three studied groups (p=0.099). Sperm concentration after treatment increased significantly when compared with before treatment in the three studied group (p=0.000) (Table II).

Sperm motility statistically significantly increased after treatment when compared with before treatment in the three studied groups (p=0.000). Percentage change of sperm motility statistically significant among the three studied groups

(p=0.000), both group A (vitamin C and zinc) and group B (Acetyle cysteine and selenium) showed statistically significant higher percentage change in sperm motility compared with group C (Vitamin E and folic acid), but there was no significant difference between group A (vitamin C and zinc) and group B (Acetyle cysteine and selenium) (Table III, Figure 2).

Normal forms of sperms statistically significantly increased after treatment when compared with before treatment in the three studied groups

(p=0.000). Percentage change of normal forms statistically significant among the three studied groups (p=0.000), both group A (vitamin C and zinc) and group B (Acetyle cysteine and selenium) showed statistically significant higher percentage change in sperm motility compared with group C (Vitamin E and folic acid), but there was no significant difference between group A (vitamin C and zinc) and group B (Acetyle cysteine and selenium) (Table IV, Figure 3).

Table (I): Age, smoking, operative and medical history among the three studied group

	Group			Test of significance (*p* value)
	Vitamin C & Zinc (n=35)	Acetyl cysteine & Selenium (n=35)	Vitamin E & Folic acid (n=35)	
Age (years)[#] - Min-Max - Median (IQR)	20.00-40.00 28.00 (25.00-31.00)	21.00-39.00 32.00 (27.00-34.00)	21.00-39.00 28.00 (23.00-33.00)	$X^2_{(KW)(df=2)}$=6.236 *p*=0.044*
Smoking	31 (88.57%)	29 (82.86%)	29 (82.86%)	$X^2_{(df=2)}$=0.590 $p_{(MC)}$=0.832 NS
Operative history - Varicocele	10 (28.57%)	5 (14.29%)	4 (11.43%)	$X^2_{(df=2)}$=3.984 $p_{(MC)}$=0.167 NS
Medical history - Diabetes mellitus	2 (5.71%)	3 (8.57%)	4 (11.43%)	$X^2_{(df=2)}$=0.729 $p_{(MC)}$=0.907 NS

IQR: Inter-quartile range (25th – 75th percentile), KW: Kruskal Wallis test, X^2: Chi-square test
df: Degree of freedom,   MC: Monte Carlo correction for Chi-Square p value. *: Statistically significant (*p*<0.05)
NS: Statistically not significant (*p*≥ 0.05),
#: Post-hoc pair-wise comparison using Dunn-Sidak method revealed insignificant difference

Table (II): Sperm concentration among the three studied group before and after treatment

	Group			Test of significance (*p* value)
	Vitamin C & Zinc (n=35)	Acetyl cysteine & Selenium (n=35)	Vitamin E & Folic acid (n=35)	
Concentration (Before) (millions) - Min-Max - Median (IQR)	5.00-11.00 9.00 (7.00-10.00)	6.00-11.00 8.00 (7.00-10.00)	6.00-10.00 9.00 (8.00-10.00)	$X^2_{(KW)(df=2)}$=0.765 *p*=0.682 NS
Concentration (After) (millions) - Min-Max - Median (IQR)	4.00-17.00 11.00 (9.00-12.00)	5.00-13.00 10.00 (8.00-12.00)	6.00-12.00 10.00 (8.00-11.00)	$X^2_{(KW)(df=2)}$=1.386 *p*=0.500 NS
Concentration (Percentage change) - Min-Max - Median (IQR)	-33.33 - 75.00 20.00 (9.09-37.50)	-22.22 - 42.86 20.00 (9.09-28.57)	-14.29 - 37.50 11.11 (0.00-20.00)	$X^2_{(KW)(df=2)}$=4.628 *p*=0.099 NS
Paired comparison Test of significance (*p* value)	$Z_{(WSR)}$=4.278 *p*=0.000*	$Z_{(WSR)}$=4.384 *p*=0.000*	$Z_{(WSR)}$=4.133 *p*=0.000*	

IQR: Inter-quartile range (25th – 75th percentile), X^2: Chi-square test, df: Degree of freedom
KW: Kruskal Wallis test, WSR: Wilcoxon Signed Ranks test, *: Statistically significant (*p*<0.05)
NS: Statistically not significant (*p*≥ 0.05)

Table (III): Sperm motility among the three studied group before and after treatment

	Group			Test of significance (*p* value)
	Vitamin C & Zinc (n=35)	**Acetyl cysteine & Selenium (n=35)**	**Vitamin E & Folic acid (n=35)**	
Motility (Before) (%) - Min-Max - Median (IQR)	4.00-29.00 20.00[a,b] (16.00-24.00)	10.00-29.00 22.00[a,b,c] (17.00-28.00)	14.00-29.00 23.00[b,c] (20.00-27.00)	$X^2_{(KW)(df=2)}$=7.155 *p*=0.028*
Motility (After) (%) - Min-Max - Median (IQR)	8.00-32.00 26.00 (17.00-29.00)	11.00-33.00 27.00 (20.00-31.00)	10.00-33.00 26.00 (19.00-30.00)	$X^2_{(KW)(df=2)}$=1.775 *p*=0.412 NS
Motility (Percentage change) - Min-Max - Median (IQR)	-20.00 - 100.00 20.83[a,b] (10.34-33.33)	-41.67 - 64.71 14.29[a,b] (8.00-21.43)	-28.57 - 40.91 6.90[c] (-5.00 - 11.76)	$X^2_{(KW)(df=2)}$=17.567 *p*=0.000*
Paired comparison Test of significance (*p* value)	$Z_{(WSR)}$=4.493 *p*=0.000*	$Z_{(WSR)}$=4.314 *p*=0.000*	$Z_{(WSR)}$=2.128 *p*=0.033*	

IQR: Inter-quartile range (25th – 75th percentile), X^2: Chi-square test, df: Degree of freedom

KW: Kruskal Wallis test, WSR: Wilcoxon Signed Ranks test, *: Statistically significant (*p*<0.05)

NS: Statistically not significant (*p*≥ 0.05)

Table (IV): Normal sperm forms among the three studied group before and after treatment

	Group			Test of significance (*p* value)
	Vitamin C & Zinc (n=35)	**Acetyl cysteine & Selenium (n=35)**	**Vitamin E & Folic acid (n=35)**	
Normal forms (Before) (%) - Min-Max - Median (IQR)	1.00-3.00 2.00 (1.00-2.00)	1.00-3.00 2.00 (1.00-3.00)	1.00-4.00 2.00 (2.00-3.00)	$X^2_{(KW)(df=2)}$=2.824 *p*=0.244 NS
Normal forms (After) (%) - Min-Max - Median (IQR)	1.00-4.00 3.00 (2.00-4.00)	1.00-4.00 3.00 (2.00-4.00)	1.00-4.00 2.00 (2.00-3.00)	$X^2_{(KW)(df=2)}$=0.506 *p*=0.777 NS
Normal forms (Percentage change) - Min-Max - Median (IQR)	-33.33 - 200.00 33.33[a,b] (0.00-100.00)	-50.00 - 100.00 33.33[a,b] (0.00-50.00)	0.00-100.00 0.00[c] (0.00-33.33)	$X^2_{(KW)(df=2)}$=12.316 *p*=0.002*
Paired comparison Test of significance (*p* value)	$Z_{(WSR)}$=4.013 *p*=0.000*	$Z_{(WSR)}$=4.041 *p*=0.000*	$Z_{(WSR)}$=2.887 *p*=0.004*	

IQR: Inter-quartile range (25th – 75th percentile), X^2: Chi-square test, df: Degree of freedom

KW: Kruskal Wallis test, WSR: Wilcoxon Signed Ranks test, *: Statistically significant (*p*<0.05)

NS: Statistically not significant (*p*≥ 0.05)

Pairwise Comparisons of Group
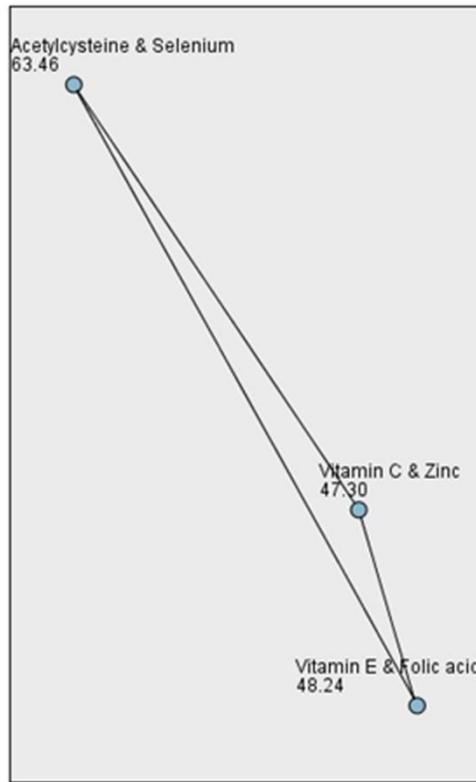
Acetylcysteine & Selenium
63.46

Vitamin C & Zinc
47.30

Vitamin E & Folic acid
48.24

Each node shows the sample average rank of Group.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Vitamin C & Zinc-Vitamin E & Folic acid	-.943	7.263	-.130	.897	1.000
Vitamin C & Zinc-Acetylcysteine & Selenium	-16.157	7.263	-2.225	.026	.078
Vitamin E & Folic acid-Acetylcysteine & Selenium	15.214	7.263	2.095	.036	.109

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Overall model among the three studied groups
$$X^2_{(KW)(df=2)} = 6.236, p = 0.044*$$

Figure (1): Pair-wise comparison of age among the three studied groups
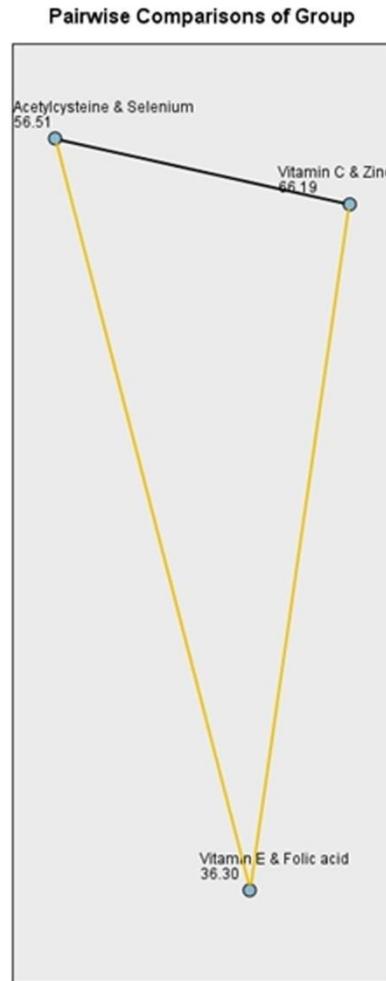
Pairwise Comparisons of Group

Each node shows the sample average rank of Group.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Vitamin E & Folic acid-Acetylcysteine & Selenium	20.214	7.277	2.778	.005	.016
Vitamin E & Folic acid-Vitamin C & Zinc	29.886	7.277	4.107	.000	.000
Acetylcysteine & Selenium-Vitamin C & Zinc	9.671	7.277	1.329	.184	.551

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Overall model among the three studied groups

$$X^2_{(KW)(df=2)}=17.567, p=0.000^*$$

Figure (2): Pair-wise comparison of percentage change of motility among the three studied groups
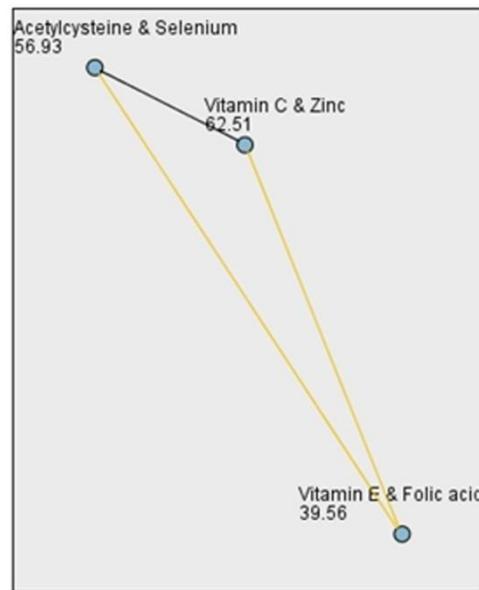
Pairwise Comparisons of Group

Each node shows the sample average rank of Group.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Vitamin E & Folic acid-Acetylcysteine & Selenium	17.371	6.822	2.546	.011	.033
Vitamin E & Folic acid-Vitamin C & Zinc	22.957	6.822	3.365	.001	.002
Acetylcysteine & Selenium-Vitamin C & Zinc	5.586	6.822	.819	.413	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Overall model among the three studied groups
$$X^2_{(KW)(df=2)}=12.316, p=0.002*$$

Figure (3): Pair-wise comparison of percentage change of normal forms of sperms among the three studied groups

Discussion

Although several clinical trials studying the role of oral antioxidants have found great improvement in the sperm parameters, but there is no doubt that general heath and special habits play an important role for the net results of the treatment.

The results of our study show that the use of combination antioxidants in infertile male with oligoasthenoteratospermia give a good results as regards sperm parameters mainly sperm concentration, sperm motility and morphology. The results of the present study are totally in line with the results of Galatioto et al.[24]

The three studied groups in the present study showed statistically significant improvement in the sperm concentration, motility and morphology, but the improvement is better among group A (Vit C + zinc) and group B (Acetyl-Cysteine + Selenium) than group C (Vit. E + folic acid) and this is in line with findings of Safarinejad et al.[15]

On the other hand, Comhaire et al., reported that N-acetyle-cysteine improved only sperm concentration without any effect on sperm morphology and motility.[25] Omu et al., found an improvement in the sperm concentration and morphology without any effect on the sperm motility in patients taking zinc as an antioxidant.[26]

Conclusion

Combination of oral antioxidant therapy is important for the improvement of one or more of sperm parameters in infertile male with oligoasthenoteratozoo-spermia. This study suggests that N-Acetyle-Cysteine and selenium are the appropriate antioxidant combination but future studies should be done on wider scale of antioxidants to choose the best to serve humanity.

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