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ORIGINAL ARTICLE – OPEN ACCESS

Duration of Oral Antioxidant Therapy in Male Infertility with Increased DNA Damage: 3 Months Versus 6 Months

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ABSTRACT

Objective: Oral antioxidants are one of the options for treating male patients with idiopathic infertility associated with increased sperm DNA fragmentation. The aim of this study is to assess the contribution of antioxidant treatment duration to treatment success in this patient group.

Materials and Methods: In this cross-sectional study (between 2014 and 2019), 637 patients who received antioxidant therapy for male infertility were retrospectively analyzed. The results of patients with 30% or more sperm DNA damage and who did not meet the exclusion criteria and who had at least 6 months of follow-up were evaluated. DNA damage, semen parameters, and laboratory results of the patients receiving antioxidant therapy were evaluated before the treatment and at the third and sixth months of treatment.

Results: A total of 53 patients with follow-up data met the study criteria. Significant decreases were observed in sperm DNA fragmentation index (DFI) values in the third and sixth months of the treatment. The sperm DFI was a median of 44% (interquartile range, 13.7%) before the treatment and 33.3% (IQR, 20.9%) after the 3 months and 18% (IQR, 13.4%) after the 6 months. Additionally, during the antioxidant treatment, a statistically significant decrease was observed between the third and sixth month DFI values.

Conclusion: In idiopathic infertility cases, antioxidant treatment may have positive effects on sperm DFI values, and the prolongation of the treatment period may make an additional contribution to treatment success for infertile men with increased sperm DNA fragmentation (SDF). Nevertheless, possible side effects cost of treatment, patient compliance, and the condition of the partner should be considered while planning the duration of treatment.

Keywords: Antioxidants, idiopathic infertility, male infertility, oxidative stress, sperm DNA damage

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INTRODUCTION

Reduction in reproductive potential due to male factor is responsible for approximately 20%–70% of all infertility cases (1). This shows the importance of evaluating the male partner in infertility. The initial evaluation, which should include a medical and reproductive history and detailed examination, should also include a conventional semen analysis. Semen analysis is not only diagnostic but also aids decision making in treatment. It has some methodological challenges despite being standardized by the World Health Organization. Although methodological difficulties reduce the potential diagnostic value of conventional semen analysis, semen analysis remains the main diagnostic test. Additionally, conventional analysis is insufficient to evaluate all cases of male infertility since it cannot fully assess functional adequacy, despite the fact that it can identify specific characteristics of sperm function (2). Studies on DNA integrity and spermatozoa fragmentation have been conducted as a result of the need for a more advanced diagnostic tool to assess male infertility and unexplained infertility (3–5). Increases in sperm DNA fragmentation (SDF) are linked to recurrent pregnancy losses in addition to having a detrimental impact on pregnancy and live birth rates (LBR) in both natural and assisted reproductive procedures (6, 7).

Oxidative stress (OS) is one of the important causes of SDF (8). Reactive oxygen species (ROS) induced by OS can impair the functions of spermatozoa at different stages (9). Additionally, it plays a key role by directly affecting DNA integrity, quality, and even the function of sperm (10). Spermatozoa are vulnerable to ROS and are also quite incapable of repairing their DNA. OS is often associated with lifestyle-related factors (e.g., smoking and alcoholism), environmental factors, varicocele, and chronic infection (11). Lifestyle changes and antioxidants can improve sperm quality by reducing the risk of SDF (12). Therefore, antioxidant agents are part of the empirical treatment. However, the data necessary to adequately support these treatments are not yet available. Thus, standard treatment protocols and durations have not been established. Hence, we retrospectively analyzed the data of the patients who applied to our clinic between 2014 and 2019 cross-sectionally. This study aims to evaluate the contribution of the duration of antioxidant therapy in infertile men with increased SDF.

Table 1. Daily antioxidant content of the patients

500 mg Vitamin C (Ester-C plus, Solgar, USA)
400 IU Vitamin E (Evicap fort, Koçak Farma, Türkiye)
600 mg N-acetyl cysteine (Assist plus, Bilim, Türkiye)
100 mcg Selenium (Selenium, Solgar, USA)
100 mg Coenzyme Q10 (Coenzyme Q-10, Solgar, USA)

MATERIALS and METHODS

The records of 637 patients who were given antioxidant treatment due to increased sperm DNA damage between 2014 and 2019 were retrospectively reviewed. Considering that this study is cross-sectional and retrospective, the sample size was not calculated and power analysis was not performed. All patients were evaluated with a baseline clinical assessment that included a comprehensive history and physical examination. Our center is a university hospital and has an andrology outpatient clinic and an assisted reproductive center. After 2–7 days of sexual abstinence, all patients made an effort to provide sperm samples at the embryology laboratory of an assisted reproductive center with the use of audiovisual stimulation. Semen samples were collected and analyzed based on the WHO criteria. Sperm DNA fragmentation index (DFI) was measured using TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling) method. The patients who were included in the study are those with a sperm DFI of 30% and above. Blood samples were taken in the morning for hormonal evaluation including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone.

Table 1 shows the agents applied by the patients. Antioxidants were selected from the medications that were found to be effective in our previous study (13). Table 2 presents the exclusion criteria of the study.

At this point, those with varicocele were excluded from the study because varicocele is an important source of oxidative stress on spermatogenesis. Those who underwent varicocelectomy were not excluded from the study, considering that these patients did not have OS.

The Başkent University Medicine and Health Sciences Research Board provided approval for this study (project number: KA19/250).

Interpretation of data and statistical analysis

Fifty-three patients met the criteria for the study and had follow-up data (Fig. 1). Age, duration of infertility, history of varicocelectomy, smoking and alcohol consumption, hormonal and seminal parameters, and sperm DFI before treatment and at 3 and 6 months of treatment were recorded.

For statistical analysis, statistical package SPSS software (v.25.0, SPSS Inc., Chicago, IL, USA) was employed. Pretreatment sperm DNA damage and the values after the third and sixth months of treatment were tested for normal distribution by the Shapiro–Wilk test, and it was found that the values did not match the normal distribution ($p<0.05$). To compare quantitative data in more than two dependent groups that did not conform to the normal distribution, the Friedman test was used. Continuous variables were defined as mean±standard deviation if normal and median if not normal. A value of $p<0.05$ was considered statistically significant. Subgroups were compared with the Bonferroni-corrected Wilcoxon test in pairs. The p-value was calculated for statistical significance with Bonferroni correction ($p=0.05/3=0.017$).

Table 2. Exclusion criteria

Varicocele
Leukocytospermia
Genetic abnormality
History of chemotherapy and/or radiotherapy
History of malignancy
History of orchiectomy and/or orchiopexy
History of hormonal therapy

Table 3. Demographic and laboratory results of patients

Age	34.4±5.3
Duration of infertility (year)	6 (1–15)
FSH (U/L)	5.34 (1.8–8.47)
LH (U/L)	5.94 (3.98–7.41)
Total testosterone (nmol/L)	3.97 (3.34–5.11)

The mean±SD was used for distributed values and the median (minimum–maximum) for non-normally distributed values. FSH: Follicle-stimulating hormone; LH: Luteinizing hormone

RESULTS

The mean age of 53 study patients was 34.4 (±5.3) years. None of the patients had concomitant health problems. There was a history of varicocelectomy in 35.8% of the patients, smoking in 35.8%, and alcohol use in 5.7%. Table 3 summarizes the FSH, LH, and total testosterone levels of the patients. Table 4 shows seminal parameters and the results of sperm DFI. There was no significant difference between the pretreatment and third and sixth month's values of semen parameters.

When the DFI values were compared before the treatment and at the third and sixth months of the treatment, it was observed that there was a significant decrease in at least one group ($\kappa^2(2)=70.89$, $p<0.001$). The median of DFI was 44% (IQR, 13.7%) before treatment but decreased to 33.3% (IQR, 20.9%) in the third month of antioxidant treatment and to 18% (IQR, 13.4%) in the sixth month. This DFI decreased between the pretreatment and third month's values ($p=0.001$), and the pretreatment and sixth month's values were statistically significant ($p<0.001$). Additionally, a statistically significant decrease in DFI values was found between the third and sixth months of antioxidant therapy ($p<0.001$). In any patient, there were no side effects that required discontinuation of antioxidant therapy.

Table 4. Results of pre-antioxidant treatment and third and sixth month's values

Semen parameters	Pretreatment	Third month	Sixth month	p
Volume (mL)	3.23 (±1.55)	3.87 (±2.12)	3.9 (±1.95)	>0.05
Concentration (million/mL)	72.02 (±71.96)	70.06 (±37.71)	75.5 (±50.97)	>0.05
Total progressive motility	47.41 (±25.02)	47.64 (±23.55)	48.3 (±26.3)	>0.05
Sperm DFI (median–IQR)	44% (13.7%)	33.3% (20.9)	18% (13.4%)	<0.001

mL: Milliliter; DFI: DNA fragmentation index; IQR: Interquartile range

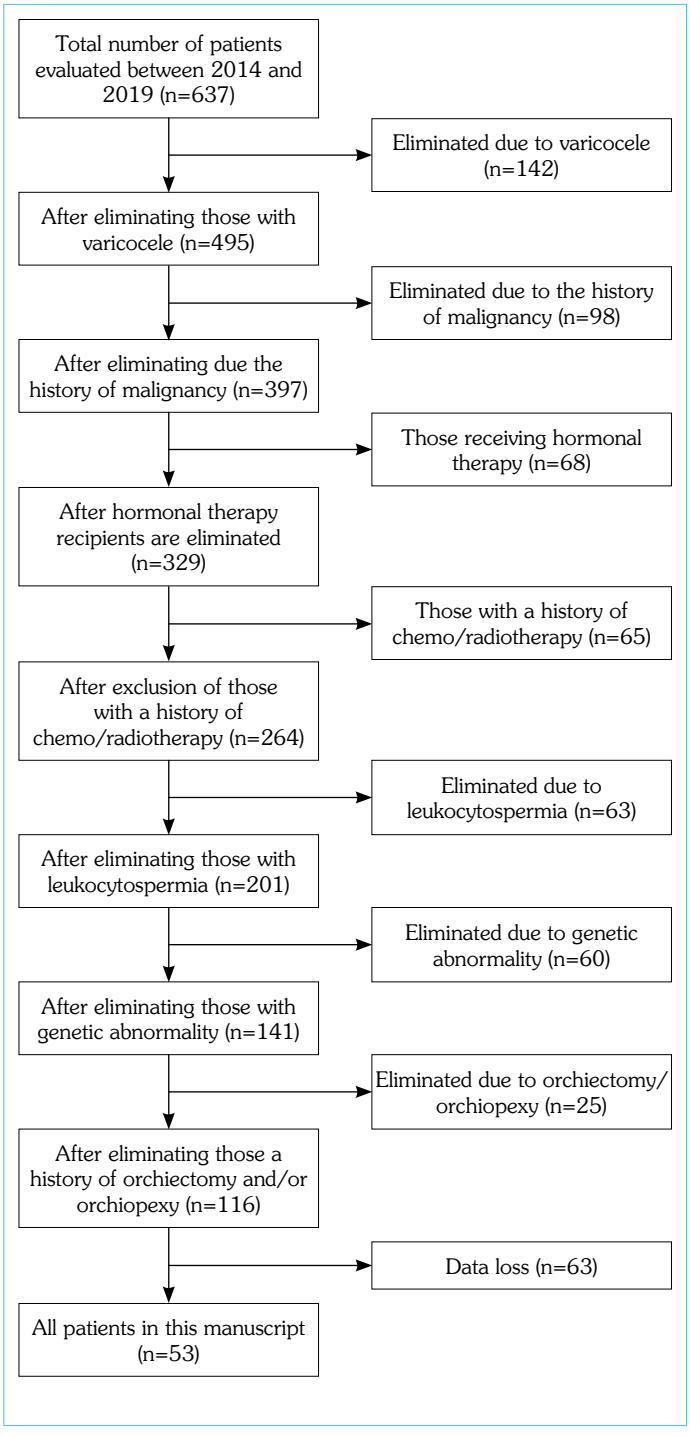

Figure 1. Flow diagram of the selected patient

DISCUSSION

SDF occurs during the spermatogenesis process, and its increase seems to be associated with impaired sperm functions and infertility. Several factors can cause sperm DNA damage, such as aging, poor lifestyle (e.g., smoking, alcohol consumption, decreased physical activity, physiological stress, and diet-related factors), environmental radiation exposure and pollution, concomitant diseases, some medications (e.g., chemotherapeutics), external genital tract infections, and presence of varicocele (8, 14, 15). These factors cause DNA breaks through OS, disruption of chromatin maturation, and apoptosis.

OS is a phenomenon that results in biomolecular oxidative damage by causing a production removal imbalance of free radicals and ROS (16, 17). The antioxidant system, alternatively, undertakes the task of neutralizing free radicals (16, 18). This protection system includes enzymatic factors and nutrients (selenium, zinc, and copper) (19–21). Deficiency of them leads to a decrease in antioxidant activity (20). This constitutes the rationale for antioxidant therapy (22, 23). Several studies support that the use of antioxidants can prevent OS-related damage and improve SDF in infertile men (21, 24).

Many oral antioxidant preparations that contain trace elements (e.g., vitamins C and E, coQ10, selenium, NAC, carnitines, zinc, pentoxifylline, and a combination of these elements) are available on the market and are often used for treatment or support in idiopathic or unexplained male infertility (23). There are several studies examining antioxidant therapy in infertile men with increased SDF. Although the majority of these studies show improvement in at least one sperm parameter, some studies show no positive contribution to the treatment success. The selection, dosage, and duration of the use of antioxidants are not clear. It is noted that when OS is present, antioxidants should be taken in higher doses. Assuming that a mature sperm development from spermatogonium is obtained in 72 ± 4 days, it is a common opinion that these agents should be used for at least 3 months (20, 25, 26). The chance of spontaneous conception declines at sperm DFI values above 20% and approaches zero for values over 30%–40% (27). Therefore, patients with a sperm DFI of 30% and above were included in the study.

In 34 randomized controlled trials using various antioxidant agents and a meta-analysis involving 2,876 couples, it has been reported that antioxidant treatment has a positive effect on LBR and pregnancy rates in assisted reproductive methods (28). In the same meta-analysis, it was also shown that this treatment caused a decrease in sperm DFI, regardless of the duration of use. The results of a recent meta-analysis involving 6,254 infertile men aged 18–65 years were similar in terms of efficacy (29). In these studies, the duration of

treatment has a big variation. Steiner's recently published The Men, Antioxidants, and Infertility study showed that antioxidant therapy only improved the concentration of seminal parameters (p=0.03) but does not affect other seminal parameters and SDF. Furthermore, the cumulative LBR could not be shown to differ between the antioxidant and placebo groups at 6 months (15% vs. 24%, p=0.14) (30).

In our study, the DFI of subfertile men (men with oligospermia) who were given antioxidant therapy and whose results could be achieved improved in the third and sixth months. Moreover, it has been shown that extending the treatment to 6 months increases the positive effect on DNA damage. However, caution should be exercised when deciding whether to extend the duration of treatment. Completing the antioxidant treatment period for 6 months in couples who do not have compliance problems and no side effects and who do not need to be given short treatment due to reasons such as partner age or decreased ovarian reserve will contribute to the acquisition of healthier sperm in terms of sperm DFI. The couple should also be informed that the contribution of this treatment to clinical reproductive outcomes is not clear. Additionally, the use of testicular sperm should be considered in couples having *in vitro* fertilization if it is not appropriate to extend the treatment duration and there is a need for sperm with less DNA damage.

SDF analysis tests also have handicaps, such as the methodological difficulties of traditional semen analysis. These methods do not have standardized threshold levels, and each method has disadvantages. Despite the fact that these tests are frequently utilized, it should be emphasized that they are still not the best methods of choice.

The most important limitations of the study include its nonrandomized and retrospective nature and its small sample size and its lack of clinical reproductive outcomes including clinical pregnancy rates and LBR. Therefore, it would be advantageous to carry out larger-scale, randomized studies that also included reproductive clinical outcomes.

CONCLUSION

Consequently, the use of oral antioxidants may help treat OS-related sperm DNA damage by reducing OS, and completing the treatment period of 6 months may make an additional contribution to treatment success for male patients with idiopathic infertility associated with increased SDF. Nevertheless, when planning the duration of treatment, possible side effects, cost of treatment, patient compliance, and the condition of the partner should be considered.

Ethics Committee Approval: The Başkent University Medicine and Health Sciences Research Board granted approval for this study (date: 23.07.2019, number: KA19/250).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – CÖ; Design – EH; Supervision – CÖ; Data Collection and/or Processing – CÖ; Analysis and/or Interpretation – EH; Literature Search – MRG; Writing – EH, MRG; Critical Reviews – CÖ.

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