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The effects of Mito-TEMPO, a mitochondria targeted antioxidant, on frozen human sperm parameters

Mitokondri hedefli bir antioksidan olan Mito-TEMPO'nun donmuş insan sperm parametreleri üzerindeki etkileri

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Abstract

Aim: The goal of this study is to find out if Mito-TEMPO, an antioxidant that targets mitochondria, can improve the quality of frozen human sperm.

Material-Method: Normospermic 25 human semen samples were frozen and stored in solutions including different concentrations (0, 5, 10 and 50 µM) of Mito-TEMPO. After thawing, they were evaluated in terms of sperm motility, viability, morphology, chromatin integrity and apoptosis.

Result: Sperm morphology was not found to change after cryopreservation. Viability was found to be significantly preserved in groups that were added Mito-TEMPO ($p<0.01$). When the motility of the sperms after they had been frozen was compared, the group that added 50 µM Mito-TEMPO had the most motile sperms. ($p<0.05$), even though there was no statistically significant difference between the groups that added 1 µM Mito-TEMPO and the groups that did not add Mito-TEMPO. In addition, chromatin decondensation and apoptosis rate decreased significantly in groups that were added Mito-TEMPO ($p<0.01$).

Conclusion: Mitochondria targeted antioxidant Mito-TEMPO improves sperm quality that decreases after thawing.

Keywords: Antioxidant, Cryopreservation, Mitochondria, Mito-TEMPO, Sperm.

Özet

Amaç: Bu çalışmanın amacı, mitokondri hedefli bir antioksidan olan Mito-TEMPO'nun donmuş insan sperm kalitesini iyileştirmedeki etkinliğini incelemektir.

Gereç ve Yöntem: Normospermik 25 insan semen örneği farklı konsantrasyonlarda (0, 5, 10 ve 50 µM) Mito-TEMPO içeren solüsyonlarda dondurularak saklandı. Çözme sonrası sperm motilitesi, viabilitesi, morfolojisi, kromatin bütünlüğü ve apoptozis bakımından değerlendirildi.

Bulgular: Kriyoprezervasyon sonrası sperm morfolojisi değişmedi. Viabilite Mito-TEMPO eklenen gruplarda önemli ölçüde korundu ($p<0.01$). Spermler kriyoprezervasyon sonrası motilite bakımından karşılaştırıldığında 1µM Mito-TEMPO eklenen grup ile Mito-TEMPO eklenmeyen grup arasında anlamlı bir farklılık gözlenmezken en fazla motil sperme rastlanan grup ise 50 µM Mito-TEMPO eklenen grup oldu ($p<0.05$). Ayrıca kromatin dekondensasyonu ve apoptozis oranı Mito-TEMPO eklenen gruplarda önemli ölçüde azaldı ($p<0.01$).

Sonuç: Mitokondri hedefli bir antioksidan olan Mito-TEMPO çözme sonrası azalan sperm kalitesini iyileştirir

Anahtar Kelimeler: Antioksidan, Kriyoprezervasyon, Mitokondri, Mito-TEMPO, Sperm.

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INTRODUCTION

Cryopreservation describes the storage of biological materials such as liquid nitrogen at cryogenic temperatures and using these when needed . Sperm freezing is an ideal solution to preserve fertility before surgical procedures as well as cytotoxic treatment such as chemotherapy and radiotherapy . Despite extensive advances in this area, Not all of the biological and biochemical parts of cryopreservation have been figured out . It is well known that at low temperatures, cell metabolism slows down, which protects germ cells, embryos, and tissues in the long run . But many things during the freezing process, like sudden changes in temperature, ice formation, and osmotic stress, have been thought to be the cause of low-quality sperm . In addition, studies have proven that cryopreservation of sperm leads to the induction of damage such as plasma membrane and mitochondrial dysfunction, DNA damage, loss of motility and viability . During the cryopreservation process, oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the production of antioxidants . Antioxidants in seminal plasma protect sperm cells from damage caused by oxidative stress. But taking out the seminal plasma, which has antioxidants, during cryopreservation lowers oxidative stress .

A large number of studies carried out recently showed that adding antioxidant during cryopreservation can neutralize ROS and improve sperm functions after freezing . However, there are few studies which show that oxidative stress is minimized by targeting ROS production sites in spermatozoa . In this regard, mitochondria, which are known as an important site of ROS production, have received great attention. Mitochondria are one of the main targets of ROS. Therefore, mitochondria targeting antioxidants have been proposed as new treatments in the last few years .

Mito-TEMPO is a compound made up of cations that like to stick to fat. It gets rid of ROS well because it has superoxide dismutase activity. It is a mix of tempo and triphenylphosphonium, which is a compound meant to get rid of superoxide in the mitochondria. It can build up about 500 times in the mitochondria and oxidizes iron ions to clean them out of the body. So, it stops the formation of hydroxyl radicals and the Fenton reaction in the oxidative chain reaction in the mitochondria. In some diseases, Mito-TEMPO has been shown to protect against damage caused by oxidation. Mito-TEMPO is known to be a good antioxidant, but it is clear that we don't know enough about how it protects sperm during cryopreservation .

The goal of this study is to find out how Mito-TEMPO, an antioxidant that works on mitochondria, affects human sperm parameters after they have been frozen.

MATERIAL and METHODS

This experiment was done on waste sperm from men between the ages of 20 and 45 who had gone to the Bolu Abant İzzet Baysal University Faculty of Medicine for a spermiogram test. The people who took part in the study were asked to sign a consent form. The study was done with permission from the Bolu Abant İzzet Baysal University Faculty of Medicine Clinical Research Ethics Committee, which gave them the number 2019/103.

Chemicals

All the chemicals were bought from Sigma Aldrich.

Preparing the semen samples

Semen samples from 25 men who had not been sexually active for 3 days were kept on a heating surface at 35°C for 30 minutes to melt them. After that, they were looked at both with a big (macroscopic) and a small (microscopic) lens (concentration, motility, viability, morphology). WHO 2020 criteria meant that normal sperm samples were part of the study (Table 1).

Table 1 Cut-off reference values for normal semen characteristics as published in consecutive WHO manuals and values in this study

PARAMETERS	WHO 2020	Values in this study
Semen volume (ml)	≥ 1.4	2.88
Sperm concentration (10^6/ml)	≥ 16 (12-16)	73.17
Total motility (PR*+NP*,%)	≥ 40 (38-42)	58.1
Viability (%)	≥54	75.1
Sperm morphology (normal form, %)	≥4.0	11.76
pH	≥7.2	≥7.2
Peroxidase-positive leukocyte (10^6/ml)	<1.0	<1.0

*PR: Progressive, *NP: Non-progressive

Sperm Cryopreservation and Thawing

After putting each sample of normal sperm in a centrifuge at 1000 rpm for 10 minutes, they were homogenized by adding 1:1 sperm washing medium (FertiPro, Belgium, Lot: FP22FL06) on the pellet obtained. After this, each of the semen samples was divided into equal volumes of cryotubes. In the first group, 3% dimethyl sulfoxide (DMSO) was added slowly to the first group as cryoprotectant and it was considered as the control group. Different concentrations (5 µM-10 µM-50 µM) of Mito-TEMPO (CAYMAN, CAS Number:1569257-94-8, USA) were added with DMSO (Sigma-Aldrich, Cat No.41639, U.S.) to the other groups, respectively. After the groups were cooled for 10 minutes in nitrogen vapour, They were put in a tank with liquid nitrogen and frozen. After 72 hours, the samples were taken out of the tank of liquid nitrogen and thawed at 37 °C for 10 minutes. To get rid of the DMSO and Mito-TEMPO, they were spun at 1000 rpm for 10 minutes. After adding 1:1 sperm washing medium to the pellet to make

it homogeneous, it was left to sit for about 30 minutes. The samples were then examined by sperm parameters.

Evaluation of Sperm Motility, Viability and Morphology

After the samples were incubated, the makler counting chamber was used to measure how active they were (Sefi Medical Instruments). WHO 2020 criteria were used to do the mobility assessment. Sperm motility was measured in terms of whether progressive, non-progressive and immotile at all. Sperm concentration was also taken into account. Eosin-Y stain was used to test sperm viability and morphology. This method is a test based on the selective permeability of the cell membrane (11). 1/1 Eosin-Y solution and the sperm sample were mixed on a slide, 200 sperm cells in different areas were counted for viability and morphology. The cells which did not receive the eosin stain were evaluated as viable and pink cells were evaluated as non-living; morphology assessment was made according to WHO 2020 criteria and viability and morphology percentage were calculated for each sample.

Chromatin Condensation Evaluation

Samples of thawed sperm were used to make smears, which were then air-dried. For acidic aniline blue staining, group smears were fixed for 10 minutes with 3% glutaraldehyde (Merck, Cat No. 8206031000, Germany). After that, they were stained with acidic aniline blue for five minutes (Carlo Erba, Cat. No. 428582, France) (pH: 3,5). After that, the specimens were rinsed in phosphate buffer solution (Phosphate Buffered Saline, PBS, Thermo Fisher Scientific, UK, pH 7.2).

Aniline blue is a standard test for finding out if sperm DNA has chromatin condensation (12). Condensed sperm with histones that are high in lysine are stained dark where the nuclei are. Light is used to stain the arginine and cysteine in the sperm's protamine nucleus (13). Under a Nikon Eclipse 80i light photomicroscope, 200 sperm cells that had been stained with acidic aniline blue were looked at. The X100 lens was used to take pictures of them.

Apoptosis Assessment

Apoptosis was done by following the instructions on the TdT mediated dUTP Nick-End Labeling (TUNEL) kits (Millipore, USA). Sperm samples were put on poly-lysine-coated slides and dried at room temperature after being spun down and washed with PBS. In a staining process that followed the instructions from the manufacturer, the preparations were left at 37 °C for 60 minutes with a TUNEL mixture, and DNA fragments were marked. DAB was passed through chromogen and alcohol series after counter staining and coated with entellan. The Nikon Eclipse 80i light photomicroscope was used to count and study 200 sperm cells in each preparation. The X100 lens was used to take pictures of them.

Statistical Analysis

Statistical analyses of the study were performed with SPSS version 26.0 analysis program. Normality distribution of the groups was evaluated with Shapiro-Wilk Test. When it was found that all of the data were not normally distributed, the data were expressed as median±IQR. Differences of data between groups were evaluated with Kruskall-Wallis Test, which is a non-parametric test. Individual group differences were evaluated with post-hoc analysis Bonferroni Test. The level of significance was set at $p \leq 0,05$.

RESULTS

This study was done with sperms from people who asked for a semen analysis. According to WHO 2020 criteria, the study included samples from people with normal sperm. The effect on sperm parameters of adding different amounts of Mito-TEMPO to solutions used for cryopreservation was studied. When Mito-TEMPO was used instead of pre-cryopreservation, the percentage of cells with normal shape didn't change. It was found that the number of viable sperm cells was much lower after cryopreservation than before ($p<0.01$). When the groups' viability after cryopreservation was compared, the Mito-TEMPO group was found to have kept its viability much better than the other groups ($p<0.01$) (Figure 1).

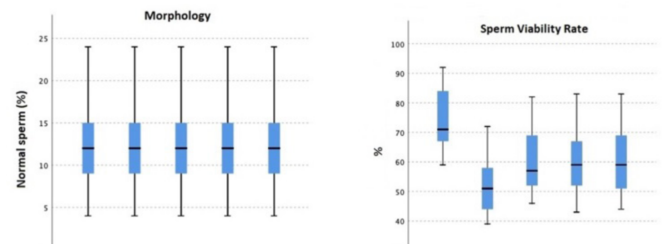

Figure 1. Pre-cryopreservation and post-cryopreservation morphology and viability values of the related groups.

When comparing the motility of all groups before and after cryopreservation, it was found that all groups had less motility after cryopreservation ($p<0.05$). When the groups were compared after cryopreservation, the highest motility loss was found in the group which was not added Mito-TEMPO ($p<0.05$). There was no significant difference between the group that got 1 µM Mito-TEMPO and the group that didn't get any, but the groups that got 10 µM and 50 µM Mito-TEMPO still moved around. The group to which 50 µM Mito-TEMPO was added had the most sperm that could move ($p<0.05$) (Figure 2).

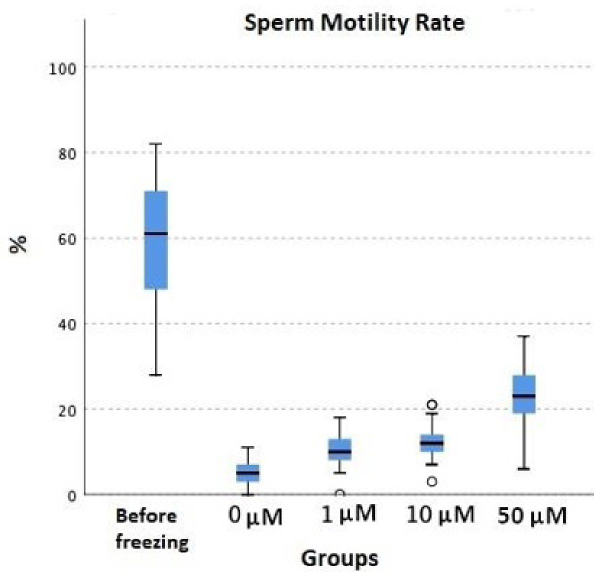

Figure 2. Pre-cryopreservation and post-cryopreservation motility values of the related groups.

When the groups were compared in terms of chromatin condensation, the rate of decondensed sperm was highest in the group that was not added Mito-TEMPO as a result of aniline blue staining ($p<0.01$) (Figure 3). When the group that was not added Mito-TEMPO and the groups that were added 1 µM and 10 µM Mito-TEMPO were compared, although fewer decondensed sperms were found in the group that was added 10 µM Mito-TEMPO, no statistical significance was found. The group closest to the chromatin condensation rate of sperm before cryopreservation was the group that was added 50 µM Mito-TEMPO ($p<0.05$) (Figure 4).

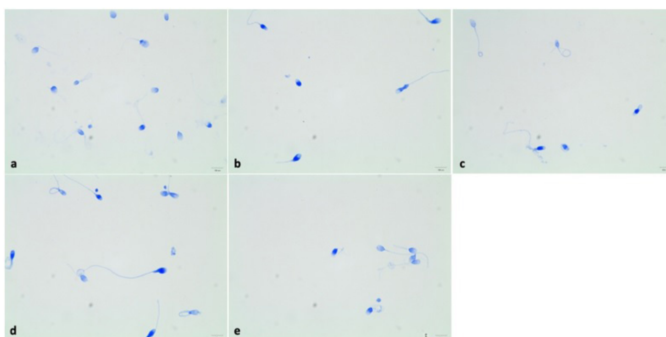

Figure 3. Pre-cryopreservation group (a), 0 µM Mito-TEMPO group (b), 1 µM Mito-TEMPO group (c), 10 µM Mito-TEMPO group (d), 50 µM Mito-TEMPO group, (d) 1000X, Scale bar: 10 µm. Condensed sperms are seen to be lightly stained with acidic aniline blue in pictures a, b, c, d and e; decondensed sperms are seen to be darkly stained with aniline blue in pictures a, b, c, d and e.

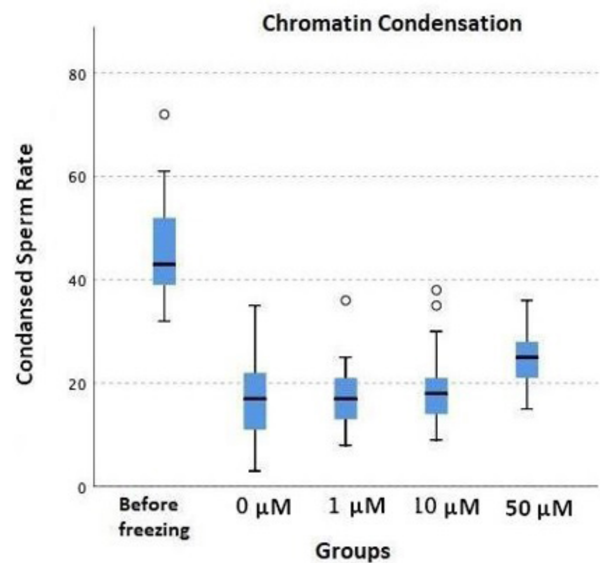

Figure 4. Pre-cryopreservation and post-cryopreservation condensed sperm values of the related groups.

When the sperm cells were compared in terms of apoptosis as a result of TUNEL staining (Figure 5), the apoptosis rate in Mito-TEMPO added groups (1µM -10µM -50µM) was statistically significantly lower than the group that was not added Mito-TEMPO ($p<0.05$) (Figure 6).

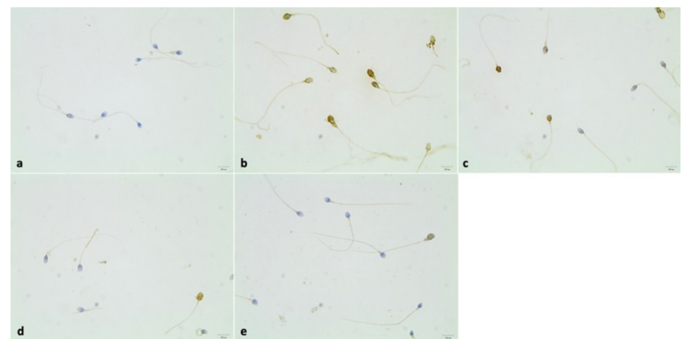

Figure 5. Pre-cryopreservation group (a), 0 µM Mito-TEMPO group (b), 1 µM Mito-TEMPO group (c), 10 µM Mito-TEMPO group (d), 50 µM Mito-TEMPO group, (d) 1000X, Scale bar: 10 µm. Apoptotic sperms are seen to be brown stained with TUNEL in pictures a, b, c, d and e; non-apoptotic sperms are seen to be blue stained with TUNEL in pictures a, b, c, d and e.

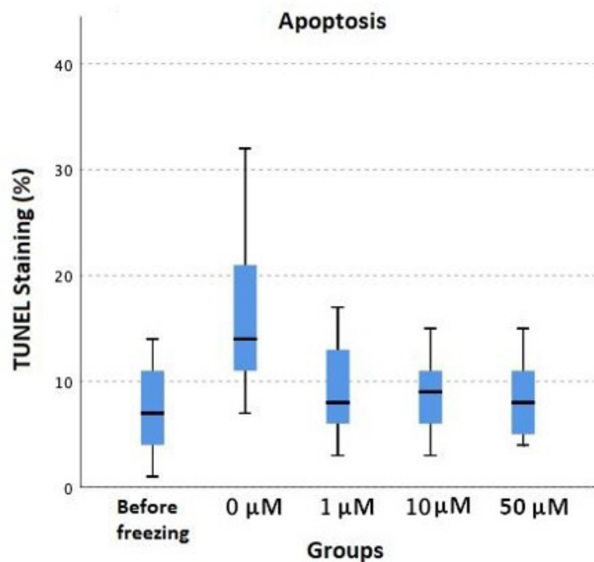

Figure 6. Pre-cryopreservation and post-cryopreservation apoptotic sperm values of the related groups.

DISCUSSION

Due to the small number of studies on how antioxidants that target mitochondria affect the freezing of sperm, this study looked at the role of different Mito-TEMPO concentrations in the freezing of human sperm. After freezing, the effects of Mito-TEMPO on the movement, shape, viability, chromatin integrity, and changes that look like apoptosis were studied. Viability and mitochondrial activity were found to decrease in sperms after freezing, and overall sperm motility was found to decrease in groups. Mito-TEMPO, on the other hand, significantly kept sperm mitochondrial activity and reduced the number of damaged mitochondria during incubation. This is likely because Mito-TEMPO goes straight to mitochondria and removes superoxide anions from mitochondria. Mito-TEMPO did not change the percentage of cells with normal shape in any of the groups. Mito-TEMPO may not have had any effect on the shape of normal and abnormal sperm because it was happened at the stage of spermatogenesis. The results of the morphology test agree with studies that say antioxidants don't change the shape of sperm in any way (10,14,15).

Cryopreservation is a key technique that is often used to keep men's ability to have children (5) and to store sperm in a stable way for a long time. Chemotherapy, radiotherapy, and surgery are all good ways to keep fertilization going before infertility interventions. Even though semen cryopreservation has made great strides in recent years, the damage caused by freezing still happens, and the quality of sperms decreases during the freezing-thawing process (16) During the cryopreservation process, oxidative stress is caused by an imbalance between ROS production and antioxidant mechanisms. Sperm cells are especially vulnerable to oxidative stress (8). When sperm is frozen, apoptotic pathways are also turned on, and ROS concentrations go up. When the amount of ROS in the body goes up, it damages the DNA of sperm and makes it harder for them to fertilize eggs. During cryopreservation, ROS are made, which changes the sperm's mitochondrial membrane potential (6). Cryopreservation changes how sperm membrane lipids are made, how long they live, and how they move. It also damages DNA in humans. Cryopreservation has a big effect on how well sperm move (17). Cryoprotectant solutions are used to lessen the stress that freezing causes. But at high concentrations, cryoprotectants are very bad for cells (4). So, cryopreservation methods need to be improved to make freezing protocol more effective (18). A lot of studies have shown that adding antioxidants to the process of freezing sperm can improve the quality of sperm that has been frozen and thawed in different ways (19–21). But there are still not enough good antioxidants (16).

Sperm endogenous antioxidants naturally neutralize ROS; however, the amount of these antioxidants decreases since seminal plasma is removed during the freezing-thawing process. For this reason, freezing medium containing supplement exogenous antioxidants is an effective method to overcome the negative effects of ROS (22,23).

While some antioxidants improve sperm functions, some may be insufficient. Antioxidants like Vitamin C, Vitamin E, catalase, quercetin, pentoxifylline, genstein, biotin, resveratrol, honey, and L-carnitine are commonly used (2).

Researchers have recently been interested in new types of antioxidants that target the mitochondria because they have many uses, work well, and don't harm the body. They have become possible ways to keep sperm from getting damaged by the stress of being frozen (24). Mitochondria is an important organelle for cell energy metabolism that modulates redox mechanism, cell development and death (9). Spermatozoa get energy for their metabolism from mitochondria through ATP synthase and oxidative phosphorylation. But they are sensitive to changes in temperature and the amount of reactive oxygen species (ROS). This makes it hard for ATP to move and lowers the quality of sperm. Its imbalance in getting rid of and making free radicals causes oxidative shock, which damages DNA and causes the cell to die (10,25). Motility, plasma membrane function, acrosome integrity, and overall viability all go down after thawing. After thawing, mobility has been said to drop from 50.6% to 30.3%. But the process by which motility goes down hasn't been fully explained yet (2).

Mito-TEMPO is an antioxidant that works on mitochondria and is a powerful ROS scavenger (26). This chemical is made by putting together tempo and triphenylphosphonium (TPP+). Tempo acts like superoxide dismutase, but while dismutase is a superoxide in the catalytic cycle, TPP is a cation that moves from the cell membrane to the cell interior (25).

This combination makes a compound that works well to remove superoxide from the mitochondria. Mito-TEMPO is also expected to keep the quality of human spermatozoa that have been frozen and then thawed (27,28). Weidinger et al. found that Mito TEMPO decreased the expression of nitric oxide synthase in the liver, as well as markers of liver and kidney damage (aspartate aminotransferase and alanine aminotransferase) (urea and creatinine) (27). Mito-Tempo is a good mitochondria-targeted antioxidant. Because it has a positive charge, it can build up 500 times in the mitochondrial matrix. It has a targeted antioxidant effect by stopping or slowing the production of mitochondrial free oxygen radicals and lipid peroxidation (24). Studies have also shown that Mito-TEMPO protects against diseases like heart damage (29), kidney damage (30), liver damage (9), Alzheimer's disease (31), sepsis models (32), Parkinson's disease (33), diabetes (34), ischemic brain disease (35), testicular toxicity (36), gastrointestinal system (37), and spinal cord damage (38).

Mito-TEMPO has been shown to improve mitochondrial function in porcine oocyte (39) and human spermatozoa (16) via decreasing oxidative stress. Seok et al. investigated the effects of different concentrations of Mito-TEMPO (0, 0,5, 5, 50 and 500 μM) on the motility of frozen-thawed porcine sperms. They proved that Mito-TEMPO addition had a beneficial effect on the motility of pig sperms (40). In another study, Kumar et al. added 50 μM Mito-TEMPO and 50 μM acetovanillone to freezing solution of buffalo sperm. When they examined semen samples in terms of progressive motility, plasma membrane integrity, lipid peroxidation, total antioxidant capacity, mitochondrial membrane potential and ROS, they reported that their separate or combined use affected the results positively (8).

In a study they conducted, Esmaeilkhanian et al. examined the efficiency of Mito-TEMPO (0, 1, 10,100 and 1000 μM) on post-thawing goat sperm quality. After thawing, they evaluated sperm mitochondria membrane potential, viability, apoptotic like changes and ROS concentration and they found that Mito-TEMPO (10 and 100 μM) improved sperm viability and reduced apoptotic like changes and ROS concentration when compared with other groups (22).

In a study they conducted on ram sperm, Zarei found that using Mito-TEMPO (0, 0.5, 5, 50 and 500 μM) improved thawed sperm motility parameters, membrane functionality, abnormal morphology, mitochondrial activity, acrosome integrity, DNA fragmentation, ROS concentration, viability and apoptotic like changes. According to the results, the efficiency of 5 and 50 μM Mito-TEMPO was found to be higher when compared with the other groups. In addition, apoptotic like changes were found to be lower in groups that were given lipid peroxidation and ROS concentration 5 and 50 μM Mito-TEMPO (10).

In a study they conducted on Verasfer variegatus, Zidni et al. froze sperms with different concentrations of Mito-TEMPO (0, 25, 50, 75, 100, 125, 150, 175 and 200 μM) and evaluated the post-thawing quality of sperms. When compared with control groups, antioxidant supplementation in sperm was found to be more effective in increasing post-thawing motility, maintaining cellular survival rates and preventing the increase in DNA damage that occurs in sperms during storing (7).

Zhang et al. found that in human sperm cryopreservation, Mito-TEMPO concentrations between 0 and 50 Mm showed significant improvement in post-thawing sperm motility, viability, membrane activity and mitochondrial membrane potential. They stated that adding Mito-TEMPO (10 and 100 μM) to cryopreservation solution improved sperm membrane integrity, mitochondrial membrane potential and chromatin integrity. They also found that Mito-TEMPO decreased the formation of oxidative stress and prevented mitochondria and DNA damage during cryopreservation (24).

In a study they conducted, Lu et al. stored semen samples by freezing them with diluents including different concentrations (0.0, 0.5, 5, 50 and 500 μM) of Mito-TEMPO. Sperm motility, viability, membrane integrity, mitochondrial membrane potential and antioxidant activities were measured. The results showed that adding Mito-TEMPO (5–50 μM) significantly increased post-thawing sperm motility, viability, membrane integrity and mitochondrial membrane potential. In the meantime, antioxidant enzyme activities increased and MDA content decreased in the group supplemented with Mito-TEMPO (16).

Asadzadeh et al. looked at the effects of Mito-TEMPO on ram sperm quality and fertility potential during the freezing-thawing process. They found that adding 5 μM and 50 μM Mito-TEMPO to semen samples led to higher post-thawing sperm motility, acrosome integrity, and viability, as well as lower lipid peroxidation and late apoptotic-like changes (25).

CONCLUSION

Using Mito-TEMPO improved deteriorated human sperm parameters during the thawing process. The damage that occurs during cryopreservation as a result of Mito-TEMPO effect can be prevented. Therefore, it can be said that adding Mito-TEMPO in diluent while thawing is an effective method in improving post-thawing sperm quality. In addition, more research is needed to investigate whether sperm fertilization, embryo implantation and pregnancy are affected. Further studies may help to clarify the mechanisms underlying protective role of Mito-TEMPO.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Analysis of the Relationship Between Serum Zinc Values and Allergic Rhinitis Parameters in Patients with Allergic Rhinitis

Alerjik Rinitli Hastalarda Serum Çinko Değerleri ile Alerjik Rinit Parametreleri Arasındaki İlişkinin Analizi

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Abstract

Background/Aim: Zinc is an significant trace element that acts an active part in the immune system and is associated with allergic inflammatory diseases. In the literature, various results have been shown in studies on the coexistence of serum zinc level and allergic rhinitis. The objective of this research was to analyze the intercourse among serum zinc levels and whole blood parameters in allergic rhinitis (AR).

Method: In this research, patients were appraised in consistency with the instruction for "Allergic Rhinitis and Its Effect on Asthma". Serum total Ig E, C-reactive protein grades in the AR group and leukocyte, neutrophil, eosinophil and lymphocyte counts, neutrophil/lymphocyte, eosinophil/lymphocyte, platelet/lymphocyte ratio and serum zinc values in whole-blood parameters of all cases were analyzed and contrasted between the AR and control groups.

Findings: Neutrophil and eosinophil counts and eosinophil/lymphocyte ratio were presented as significantly superior in the AR category than in the control category ($p<0.05$). Serum zinc values in the AR category were remarkably underneath than those in the control category ($p<0.05$). In the univariate model, it was noticed that the eosinophil and zinc values had a significant-independent differential effect in distinguishing the AR and control category patients ($p< 0.05$).

Conclusion: Serum zinc level, neutrophil and eosinophil count, eosinophil/lymphocyte ratio in the blood are practical biochemical indicators that can be used to recognition, treatment, together with following-up of cases with allergic rhinitis. It is assumed that with the support of zinc deficiency, important clinical benefits can be achieved in anti-allergic treatment.

Keywords: Rhinitis, allergic, trace elements, skin tests

Özet

Giriş/Amaç: Çinko, bağışıklık sisteminde aktif rol oynayan, alerjik inflamatuar hastalıklarla ilişkili önemli bir eser elementtir. Literatürde serum çinko düzeyi ile alerjik rinit hastalığının birlikteliği konusunda yapılan çalışmalarda farklı sonuçlar gösterilmiştir. Bu çalışmanın amacı, alerjik rinitte serum çinko seviyeleri ile tam kan değerleri arasındaki ilişkiyi araştırmaktır.

Yöntem: Alerjik rinit tanısı alan erişkin yüz otuz iki kişi hasta ve yüz otuz altı kişi sağlıklı kontrol grubu olarak incelenmiştir. Hastalar "Alerjik Rinit ve Astım Üzerindeki Etkisi" kılavuzuna göre değerlendirilmiştir. Hasta grubunda serum total IgE, C reaktif protein düzeyleri ile tüm olguların tam kan parametrelerinde lökosit, nötrofil, eozinofil ve lenfosit sayıları, nötrofil lenfosit, eozinofil lenfosit, platelet lenfosit oranı ve serum çinko değerleri incelenmiş, hasta ve kontrol grubu arasında karşılaştırılmıştır.

Bulgular: Hasta grubunda nötrofil ve eozinofil sayısı, eozinofil lenfosit oranı kontrol grubundan kayda değer ölçüde daha yüksek bulunmuştur ($p<0.05$). Hasta grubunda serum çinko değeri kontrol grubundan anlamlı olarak daha az tespit edilmiştir ($p<0.05$). Tek değişkenli modelde vaka ve kontrol grubu hastalarını ayırmada eozinofil, çinko değerinin anlamlı-bağımsız ayırıcı etkisi olduğu gözlenmiştir ($p< 0.05$).

Sonuç: Çinko, alerjik rinitte inflamasyonda rol alan önemli bir eser elementtir. Serum çinko düzeyi, kanda nötrofil ve eozinofil sayısı, eozinofil lenfosit oranı alerjik rinit hastalarında tanı, tedavi ve takipte kullanılabilecek pratik biyokimyasal göstergelerdir. Çinko eksikliğinin desteklenmesi ile anti alerjik tedavide önemli klinik faydalar sağlanılabileceği düşünülmektedir.

Türkçe Anahtar Kelimeler : Rinit, alerjik, eser elementler, deri testleri

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INTRODUCTION

Allergic rhinitis (AR) is an important global health problem. AR is one of the common complaints for applying to the otolaryngology (ENT) outpatient clinic. The mean prevalence of AR is about 18.1% (1%-54.5%) in the general population (1, 2).

It is known that systemic inflammation takes place in addition to nasal inflammation in AR pathogenesis. It is characterized by local collection of inflammatory cells such as; T-lymphocyte, mast cells, eosinophils, basophils, besides neutrophils in blood and tissue (3). AR results from immunoglobulin-E (IgE)-mediated reactions to inhaled allergens. It is a common chronic disease worldwide (4).

AR has a negative effect on daily activity, school performance, and academic achievement. It is also known to reduce the quality of life by causing a loss of workforce and sleep disorders (5).

The main symptoms of AR are rhinorrhea, nasal itching, sneezing, and nasal congestion. Rhinorrhea is usually profuse and serous in nature. Paroxysmal sneezing attacks are the most characteristic symptoms of AR and are accompanied by nasal itching and irritation (6). In the Allergic Rhinitis and its Impact on Asthma (ARIA) guideline, AR is classes as intermittent or persistent and mild or moderate/severe. Determination is bottomed on the practical records together with physical examination. In cases who have uncontrollable rhinitis or long-term symptoms despite medications, skin prick tests to identify the allergen or the presence of specific IgE antibodies against the allergen should be examined (4).

IgE has a main act in type I hypersensitivity, which reflects the sensitivity of mast cells by allergen-specific IgE antibodies bound to their high-affinity receptors (FcεRI) (7). Zinc (Zn) has numerous physiological functions. It is an prominent trace element that performs an significant act in the immune tract. Zn influences many perspectives of immune task, including thymic improvement and the actions of immune cells. Zn is also related in several steps of FceRI-induced mast cell activation required for degranulation and cytokine production. It has been indicated to impede the production and mRNA expression of inflammatory cytokines. Zn is known to task as an antioxidant and stabilize cell membranes. It has been stated that Zn has an important function in allergic inflammation (8, 9). This relationship among reduced Zn levels and asthma and atopic dermatitis has been more clearly demonstrated (10, 11).

Measurement of complete blood count parameters in AR patients is cost-effective and easy to perform. Recently, there has been a tendency to use eosinophil, lymphocyte, and neutrophil ratios instead of numerical values. Neutrophil/lymphocyte ratio (NLR), eosinophil/lymphocyte ratio (ELR), and platelet/lymphocyte ratio (PLR) can be computed easily (12).

Few studies have been directed on the role of mark elements in AR disease together with they have reported contradictory outcomes. Since different results have been reported in various studies, further studies are needed. In the textes, the number of studies on the relationship among serum total IgE and Zn levels is very scarce, and there is no study on the correlation between NLR, ELR and PLR, and Zn levels.

This research aims to investigate the intercourse among serum Zn and serum total IgE grades, blood neutrophil, eosinophil and lymphocyte counts, NLR, ELR, and PLR in AR.

MATERIAL and METHODS

Study Design

Between January 2021 and September 2022, 132 patients who asked to the outpatient clinic of the Faculty of Medicine Hospital, Department of Otorhinolaryngology were contained in the investigation. Cases over the age of 18 who were diagnosed with AR and presented to the ENT outpatient clinic with objections of itching in the nose and palate, nasal congestion, nasal discharge and sneezing, whose symptoms lasted more than four weeks, and more often than four days a week and did not receive any medical treatment, were evaluated. Errors were determined according to ARIA guidelines.

In the AR group, skin prick test results, C-reactive protein (CRP) levels, serum total IgE and blood parameters of leukocyte, neutrophil, eosinophil and lymphocyte counts, NLR, ELR, PLR and serum Zn values were recorded. NLR, ELR and PLR were calculated by dividing neutrophils, eosinophils and platelets by the percentage of lymphocytes in the complete blood count analysis.

One hundred thirty-six people of similar age groups and genders who did not have AR symptoms and who were received to the medical institution due to other complaints were joined in the research as the control group.

Patients in the pediatric age group (<18) and who were recently (less than 4 weeks ago) diagnosed with AR, with a history of nasal surgery, nasal polyps, oncological diagnosis, and pregnant cases were excepted from the research.

The investigation protocol was managed in pursuance with the ethical principles in the Declaration of Helsinki and was approved by the institutional ethics committee (approval date/number 19.10.2022/10-02). The authors declared that they followed the protocols used in the study centers regarding the publication of patient data.

Zinc Levels

Serum Zn levels were measured with the brand commercial kit (Archem Diagnostics (İstanbul, TURKEY)) using colorimetric method defined in the literature. (13).

Statistical analysis

Mean, standard deviation, median minimum, maximum, frequency and ratio values were used as descriptive statistics. The distribution of variables was analyzed with the Kolmogorov-Smirnov test. The Mann-Whitney u test (in analysis of quantitative independent data), the Chi-square test (in analysis of qualitative independent data) were used. ROC curve was used to investigate the effect level and cut-off value. The effect level was analyzed with univariate and multivariate logistic regression. SPSS 28.0 (IBM Corp. Armonk NY) program was used in the analysis.

RESULTS

Patient characteristics

In the study, 132 cases with AR besides 136 control cases were evaluated. The average age was 35.5 ± 12.5 in the AR group, and 38.9 ± 14.8 in the control group. The female/male ratio was 91/41 in the AR, besides 91/45 in the control group. There was not any statistically meannig ($p > 0.05$) difference with the age and gender dispersion of the cases between the AR and control group.

The skin prick test involved positive control, negative control, tree mix, olea europeae (olive tree), cockroach, cat epithelium, weed mix, weed mix, mold mix II and mite mix allergens. Single allergen positivity was interpreted as monoallergic, and multiple allergen positivity was interpreted as polyallergic condition. The outcomes of the derm prick assays revealed 31 monoallergic and 35 polyallergic cases. The prick test was negatory in 66 cases.

With regard to the biochemistry parameters, platelet and lymphocyte values, NLR and PLR values did not differ statistically between the AR and control group ($p > 0.05$).

Neutrophil and eosinophil counts and ELR values in the AR category were statistically remarkably ($p < 0.05$) superior than the control category. Elevated serum total-IgE levels were described as > 100 kU/L. Serum total IgE was elevated in 37 patients and was within normal limits in 95 cases. The findings are represented in tables 1 and 2.

Effect of serum Zn levels on the possibility of allergic sensitization

Serum Zn values in the AR category were statistically remarkably lower than the control category ($p < 0.05$) (Table 2, Figure 1). In the univariate model, a statistically significant ($p < 0.05$) differential effect of neutrophil, eosinophil, ELR and Zn values in differentiating the cases in AR and control group was observed, while a significant-independent ($p < 0.05$) differential effect of eosinophil and Zn values was observed in the multivariate model (Table 3).

The mean serum Zn level was 81.6±14.3. There is a significant difference in serum Zn levels among the cases in the AR and control groups [The ROC analysis; Area under the curve 0.758 (0.701-0.816)], and the cut-off value was obtained as 72.6 [Area under the curve 0.694 (0.630-0.758)]. (sensitivity: 93.4%, positive prediction: 45.5%, specificity: 63.8%, and negative prediction :75.8%) (Table 4).

Relationship between serum zinc level and allergic sensitivity

Platelet, lymphocyte, eosinophil counts and ELR, PLR and CRP values didn't differ statistically between the groups with serum Zn levels < 72.6 and ≥ 72.6 ($p > 0.05$). Neutrophil counts and NLR were remarkably higher ($p < 0.05$) in the category with serum Zn level < 72.6 than in the group with ≥ 72.6. In the group with serum Zn level < 72.6, the derm prick test and serum total IgE positivity rate were statistically higher ($p < 0.05$) (Table 5).

DISCUSSION

Low serum Zn has been related with various chronic diseases. There are a few studies examining the association among Zn levels together with allergic diseases, like allergic asthma and atopic dermatitis (14). However, there are studies with different results to explain the relationship (15).

In various studies conducted in adult and pediatric age groups, AR patients and healthy control groups were compared and it was shown that serum Zn was significantly lower in the AR patient group (16-18). Conversely, there are also works demonstrations that there is no significant distinction (15).

In our research, serum Zn values were determined to be significantly under in the AR category compared to the control category.

It has been determined in various investigations that Zn has an significant role in allergic inflammatory diseases, and it has been manifested to play a act in distinct IgE-related cellular signaling cascades (19). It has been statemented that there is an imbalance in IgE-dependent immunological activities in the presence of low Zn (19). Seo et al. investigated the relationship between total IgE and serum zinc levels in AR patients and showed that serum zinc grades were low in cases with elevated serum total IgE levels, and there was a negative linear correlation between total IgE levels and zinc levels (20).

In our research, it was noticed that the total IgE level was significantly superior in the group with serum Zn level < 72.6 contrasted to the group with Zn level ≥ 72.6. Our findings promoted the investigations in the written works.

Neutrophilia is one of the systemic inflammatory markers and is associated with inflammatory diseases (21). Eosinophilic inflammation is a common feature in allergic diseases. The eosinophil count is used to state the intensity of allergic diseases. In their research, Li et al. statemented that the eosinophil count and serum total IgE level were higher in patients with AR (22).

NLR and ELR are simple biochemical parameters that can be easily performed in the detection of inflammatory and infectious illnesses (21, 23). Yenigün et al. showed that NLR and ELR values increased as a result of the systemic inflammatory reply in nasal polyposis disease (21). In their study on children with functional dyspepsia, an inflammatory disease, Savas et al. pointed out a statistically significant distinction in NLR values between the control and the AR group. Although there was numerical distinction in serum Zn levels among the groups, it was not statistically significant (24).

In a research by Kant et al., eosinophil counts and ELR quantities were noticed to be remarkably higher and NLR values lower in AR cases matched to the healthful ones (25). Göker et al. found higher NLR and PLR values in AR patients matched to the healthful group (26).

In our investigation, neutrophil and eosinophil levels were higher in the AR group. Serum Zn levels and ELR values were found to be effective in differentiating the AR and control groups. In multivariate logistic regression analysis, eosinophil count and Zn levels were found as significant independent discriminative variables. These results support that Zn levels and eosinophilia are parameters associated with AR.

This investigation has some limitations. It is uncertain whether a individual evaluation of serum Zn level exactly matches the actual serum Zn level in the population.

Since it was not checked whether there was an improvement in AR patients after Zn deficiency treatment, obvious information could not be acquired about the role of Zn deficiency in the intensity and treatment of the disease.

Knowledge on the act of serum total IgE as a marker in AR intensity and diagnosis is restricted and changeable. This issue should be addressed further.

Failure to look at the correlation of allergen-specific IgE with Zn level created a limitation in the specificity of the study.

CONCLUSION

Zn is a crucial trace element involved in the definition of the severity and therapy of chronic inflammatory diseases. The low serum Zn levels detected in AR patients in our study support the facts of other investigations in the literary texts. Morover, the association between neutrophilia, eosinophilia, high ELR, low Zn levels and AR indicates that a low Zn level might be associated with AR disease. Checking the serum Zn concentration is beneficial in the aftercare and therapy of AR disease, and we trust that it may be a useful strategy for advancing anti-allergy treatments.

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TABLES

Table 1. Demographic data of the entire cohort

		Min-Max	Median	Mean ± sd/ n (%)
Age		18.0 - 84.0	34.0	37.2 ± 13.8
Gender	Female			182 (67.9%)
	Male			86 (32.1%)
Platelet (x10³)		79.0 - 532.0	272.0	279.7 ± 68.8
Neutrophil (x10³)		0.97 - 9.00	3.89	4.13 ± 1.38
Lymphocyte (x10³)		0.78 - 42.50	2.22	2.56 ± 2.75
Eosinophil (x10³)		0.00 - 1.85	0.16	0.22 ± 0.21
NLR		0.58 - 6.81	1.70	1.88 ± 0.79
ELR		0.00 - 0.95	0.07	0.10 ± 0.10
PLR		1.0 - 475.2	119.9	125.1 ± 45.9
CRP		0.10 - 2.00	0.10	0.19 ± 0.25
Zinc		43.2 - 143.8	79.9	81.6 ± 14.3
Prick Test	Non			66 (50.0%)
	Mono			31 (23.5%)
	Poly			35 (26.5%)
Total IGE	High			37 (28.0%)
	Normal			95 (72.0%)
Rhinitis				132 (49.3%)
Healthy controls				136 (50.7%)

Table 2. Comparison of AR and control groups

		AR Group		Control Group		p
		Mean ± sd/ n(%)	Median	Mean ± sd/ n (%)	Median	
Age		35.5 ± 12.5	34.0	38.9 ± 14.8	35.5	0.080 [m]
Gender	Female	91 (68.9%)		91 (66.9%)		0.722 [X^2]
	Male	41 (31.1%)		45 (33.1%)		
Platelet (x10³)		286.5 ± 63.9	276.5	273.0 ± 72.7	265.0	0.052 [m]
Neutrophil (x10³)		4.32 ± 1.35	4.10	3.94 ± 1.40	3.62	***0.013*** [m]
Lymphocyte (x10³)		2.67 ± 3.55	2.29	2.45 ± 1.63	2.19	0.363 [m]
Eosinophil (x10³)		0.26 ± 0.25	0.17	0.18 ± 0.15	0.15	***0.003*** [m]
NLR		1.92 ± 0.71	1.75	1.84 ± 0.87	1.60	0.102 [m]
ELR		0.11 ± 0.12	0.08	0.08 ± 0.07	0.06	***0.005*** [m]
PLR		124.2 ± 38.8	119.6	125.9 ± 52.0	120.1	0.530 [m]
Zinc		75.7 ± 12.8	74.1	87.4 ± 13.4	86.8	***<0.001*** [m]

[m] Mann-Whitney u test / [X^2] Chi-Square test

Table 3. Evaluation of the effectiveness of laboratory parameters with Univariate and Multivariate analysis

	Univariate Model			Multivariate Model		
	OR	%95 CI	p	OR	%95 CI	p
Neutrophil	1.22	1.02 - 1.46	***0.027***			
Eosinophil	10.76	2.33 - 49.63	***0.002***	11.21	2.14 - 58.65	***0.004***
ELR	58.22	2.42 - 1403.7	***0.012***			
Zinc	0.928	0.906 - 0.950	***<0.001***	0.927	0.904 - 0.950	***<0.001***

Logistic Regression (Forward LR)

Table 4. Evaluation of the effectiveness of serum zinc level in separating AR and control groups by ROC analysis

	Area under the curve	%95 Confidence Interval	p
Zinc	0.758	0.701 - 0.816	***<0.001***
Zinc Cut Off Value 72.6	0.694	0.630 - 0.758	***<0.001***

		Control Group	AR Group		%
Zinc	< 72.6	60	9	Sensitivity	93.4%
	≥ 72.6	72	127	Positive Prediction	45.5%
				Specificity	63.8%
				Negative Prediction	87.0%

Table 5. Comparison of AR patients according to serum zinc ≥ 72.6 cut off value

		Zinc < 72.6		Zinc ≥ 72.6		p
		Mean ± sd/ n (%)	Median	Mean ± sd/ n (%)	Median	
Age		36.2 ± 14.2	33.0	37.6 ± 13.7	35.0	0.326 [m]
Gender	Female	55 (79.7%)		127 (63.8%)		***0.015*** [X^2]
	Male	14 (20.3%)		72 (36.2%)		
Platelet (x10³)		286.3 ± 70.1	273.0	277.4 ± 68.3	271.0	0.333 [m]
Neutrophil (x10³)		4.60 ± 1.33	4.40	3.97 ± 1.37	3.69	***<0.001*** [m]
Lymphocyte (x10³)		2.25 ± 0.59	2.19	2.66 ± 3.16	2.25	0.341 [m]
Eosinophil (x10³)		0.24 ± 0.23	0.16	0.21 ± 0.21	0.16	0.892 [m]
NLR		2.19 ± 0.96	1.95	1.77 ± 0.69	1.60	***<0.001*** [m]
ELR		0.11 ± 0.10	0.07	0.09 ± 0.10	0.07	0.469 [m]
PLR		134.7 ± 53.9	126.0	121.7 ± 42.4	117.0	0.062 [m]
CRP		0.20 ± 0.30	0.10	0.18 ± 0.20	0.10	0.666 [m]
Zinc		65.5 ± 5.7	66.1	87.2 ± 12.0	85.1	
Prick Test	Non	20 (33.3%)		46 (63.9%)		***0.001*** [X^2]
	Mono	22 (36.7%)		9 (12.5%)		
	Poly	18 (30.0%)		17 (23.6%)		
Total IgE	(+)	25 (41.7%)		12 (16.7%)		***0.001*** [X^2]
	(-)	35 (58.3%)		60 (83.3%)		

FIGURES

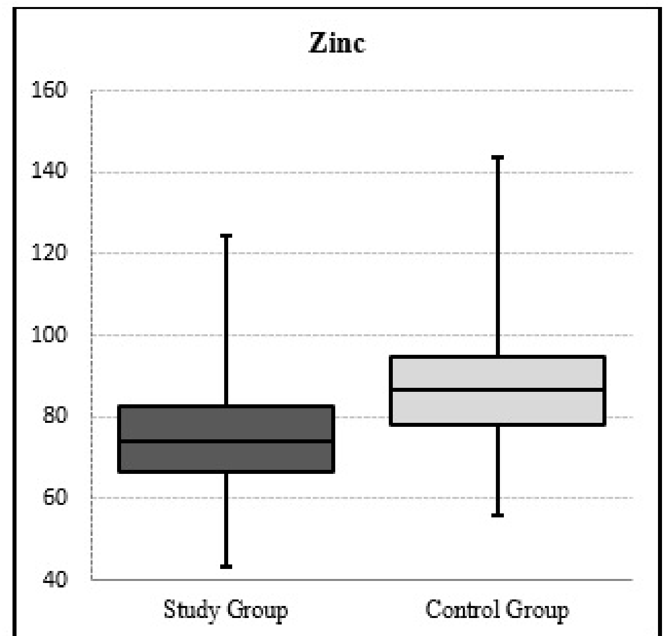

Figure 1. Evaluation of the effectiveness of serum zinc level in separating AR and control groups by ROC Cure

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The effects of Mito-TEMPO, a mitochondria targeted antioxidant, on frozen human sperm parameters

Mitokondri hedefli bir antioksidan olan Mito-TEMPO'nun donmuş insan sperm parametreleri üzerindeki etkileri

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Abstract

Aim: The goal of this study is to find out if Mito-TEMPO, an antioxidant that targets mitochondria, can improve the quality of frozen human sperm.

Material-Method: Normospermic 25 human semen samples were frozen and stored in solutions including different concentrations (0, 5, 10 and 50 µM) of Mito-TEMPO. After thawing, they were evaluated in terms of sperm motility, viability, morphology, chromatin integrity and apoptosis.

Result: Sperm morphology was not found to change after cryopreservation. Viability was found to be significantly preserved in groups that were added Mito-TEMPO ($p<0.01$). When the motility of the sperms after they had been frozen was compared, the group that added 50 µM Mito-TEMPO had the most motile sperms. ($p<0.05$), even though there was no statistically significant difference between the groups that added 1 µM Mito-TEMPO and the groups that did not add Mito-TEMPO. In addition, chromatin decondensation and apoptosis rate decreased significantly in groups that were added Mito-TEMPO ($p<0.01$).

Conclusion: Mitochondria targeted antioxidant Mito-TEMPO improves sperm quality that decreases after thawing.

Keywords: Antioxidant, Cryopreservation, Mitochondria, Mito-TEMPO, Sperm.

Özet

Amaç: Bu çalışmanın amacı, mitokondri hedefli bir antioksidan olan Mito-TEMPO'nun donmuş insan sperm kalitesini iyileştirmedeki etkinliğini incelemektir.

Gereç ve Yöntem: Normospermik 25 insan semen örneği farklı konsantrasyonlarda (0, 5, 10 ve 50 µM) Mito-TEMPO içeren solüsyonlarda dondurularak saklandı. Çözme sonrası sperm motilitesi, viabilitesi, morfolojisi, kromatin bütünlüğü ve apoptozis bakımından değerlendirildi.

Bulgular: Kriyoprezervasyon sonrası sperm morfolojisi değişmedi. Viabilite Mito-TEMPO eklenen gruplarda önemli ölçüde korundu ($p<0.01$). Spermler kriyoprezervasyon sonrası motilite bakımından karşılaştırıldığında 1µM Mito-TEMPO eklenen grup ile Mito-TEMPO eklenmeyen grup arasında anlamlı bir farklılık gözlenmezken en fazla motil sperme rastlanan grup ise 50 µM Mito-TEMPO eklenen grup oldu ($p<0.05$). Ayrıca kromatin dekondensasyonu ve apoptozis oranı Mito-TEMPO eklenen gruplarda önemli ölçüde azaldı ($p<0.01$).

Sonuç: Mitokondri hedefli bir antioksidan olan Mito-TEMPO çözme sonrası azalan sperm kalitesini iyileştirir

Anahtar Kelimeler: Antioksidan, Kriyoprezervasyon, Mitokondri, Mito-TEMPO, Sperm.

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INTRODUCTION

Cryopreservation describes the storage of biological materials such as liquid nitrogen at cryogenic temperatures and using these when needed . Sperm freezing is an ideal solution to preserve fertility before surgical procedures as well as cytotoxic treatment such as chemotherapy and radiotherapy . Despite extensive advances in this area, Not all of the biological and biochemical parts of cryopreservation have been figured out . It is well known that at low temperatures, cell metabolism slows down, which protects germ cells, embryos, and tissues in the long run . But many things during the freezing process, like sudden changes in temperature, ice formation, and osmotic stress, have been thought to be the cause of low-quality sperm . In addition, studies have proven that cryopreservation of sperm leads to the induction of damage such as plasma membrane and mitochondrial dysfunction, DNA damage, loss of motility and viability . During the cryopreservation process, oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the production of antioxidants . Antioxidants in seminal plasma protect sperm cells from damage caused by oxidative stress. But taking out the seminal plasma, which has antioxidants, during cryopreservation lowers oxidative stress .

A large number of studies carried out recently showed that adding antioxidant during cryopreservation can neutralize ROS and improve sperm functions after freezing . However, there are few studies which show that oxidative stress is minimized by targeting ROS production sites in spermatozoa . In this regard, mitochondria, which are known as an important site of ROS production, have received great attention. Mitochondria are one of the main targets of ROS. Therefore, mitochondria targeting antioxidants have been proposed as new treatments in the last few years .

Mito-TEMPO is a compound made up of cations that like to stick to fat. It gets rid of ROS well because it has superoxide dismutase activity. It is a mix of tempo and triphenylphosphonium, which is a compound meant to get rid of superoxide in the mitochondria. It can build up about 500 times in the mitochondria and oxidizes iron ions to clean them out of the body. So, it stops the formation of hydroxyl radicals and the Fenton reaction in the oxidative chain reaction in the mitochondria. In some diseases, Mito-TEMPO has been shown to protect against damage caused by oxidation. Mito-TEMPO is known to be a good antioxidant, but it is clear that we don't know enough about how it protects sperm during cryopreservation .

The goal of this study is to find out how Mito-TEMPO, an antioxidant that works on mitochondria, affects human sperm parameters after they have been frozen.

MATERIAL and METHODS

This experiment was done on waste sperm from men between the ages of 20 and 45 who had gone to the Bolu Abant İzzet Baysal University Faculty of Medicine for a spermiogram test. The people who took part in the study were asked to sign a consent form. The study was done with permission from the Bolu Abant İzzet Baysal University Faculty of Medicine Clinical Research Ethics Committee, which gave them the number 2019/103.

Chemicals

All the chemicals were bought from Sigma Aldrich.

Preparing the semen samples

Semen samples from 25 men who had not been sexually active for 3 days were kept on a heating surface at 35°C for 30 minutes to melt them. After that, they were looked at both with a big (macroscopic) and a small (microscopic) lens (concentration, motility, viability, morphology). WHO 2020 criteria meant that normal sperm samples were part of the study (Table 1).

Table 1 Cut-off reference values for normal semen characteristics as published in consecutive WHO manuals and values in this study

PARAMETERS	WHO 2020	Values in this study
Semen volume (ml)	≥ 1.4	2.88
Sperm concentration (10^6/ml)	≥ 16 (12-16)	73.17
Total motility (PR*+NP*,%)	≥ 40 (38-42)	58.1
Viability (%)	≥54	75.1
Sperm morphology (normal form, %)	≥4.0	11.76
pH	≥7.2	≥7.2
Peroxidase-positive leukocyte (10^6/ml)	<1.0	<1.0

*PR: Progressive, *NP: Non-progressive

Sperm Cryopreservation and Thawing

After putting each sample of normal sperm in a centrifuge at 1000 rpm for 10 minutes, they were homogenized by adding 1:1 sperm washing medium (FertiPro, Belgium, Lot: FP22FL06) on the pellet obtained. After this, each of the semen samples was divided into equal volumes of cryotubes. In the first group, 3% dimethyl sulfoxide (DMSO) was added slowly to the first group as cryoprotectant and it was considered as the control group. Different concentrations (5 µM-10 µM-50 µM) of Mito-TEMPO (CAYMAN, CAS Number:1569257-94-8, USA) were added with DMSO (Sigma-Aldrich, Cat No.41639, U.S.) to the other groups, respectively. After the groups were cooled for 10 minutes in nitrogen vapour, They were put in a tank with liquid nitrogen and frozen. After 72 hours, the samples were taken out of the tank of liquid nitrogen and thawed at 37 °C for 10 minutes. To get rid of the DMSO and Mito-TEMPO, they were spun at 1000 rpm for 10 minutes. After adding 1:1 sperm washing medium to the pellet to make

it homogeneous, it was left to sit for about 30 minutes. The samples were then examined by sperm parameters.

Evaluation of Sperm Motility, Viability and Morphology

After the samples were incubated, the makler counting chamber was used to measure how active they were (Sefi Medical Instruments). WHO 2020 criteria were used to do the mobility assessment. Sperm motility was measured in terms of whether progressive, non-progressive and immotile at all. Sperm concentration was also taken into account. Eosin-Y stain was used to test sperm viability and morphology. This method is a test based on the selective permeability of the cell membrane (11). 1/1 Eosin-Y solution and the sperm sample were mixed on a slide, 200 sperm cells in different areas were counted for viability and morphology. The cells which did not receive the eosin stain were evaluated as viable and pink cells were evaluated as non-living; morphology assessment was made according to WHO 2020 criteria and viability and morphology percentage were calculated for each sample.

Chromatin Condensation Evaluation

Samples of thawed sperm were used to make smears, which were then air-dried. For acidic aniline blue staining, group smears were fixed for 10 minutes with 3% glutaraldehyde (Merck, Cat No. 8206031000, Germany). After that, they were stained with acidic aniline blue for five minutes (Carlo Erba, Cat. No. 428582, France) (pH: 3,5). After that, the specimens were rinsed in phosphate buffer solution (Phosphate Buffered Saline, PBS, Thermo Fisher Scientific, UK, pH 7.2).

Aniline blue is a standard test for finding out if sperm DNA has chromatin condensation (12). Condensed sperm with histones that are high in lysine are stained dark where the nuclei are. Light is used to stain the arginine and cysteine in the sperm's protamine nucleus (13). Under a Nikon Eclipse 80i light photomicroscope, 200 sperm cells that had been stained with acidic aniline blue were looked at. The X100 lens was used to take pictures of them.

Apoptosis Assessment

Apoptosis was done by following the instructions on the TdT mediated dUTP Nick-End Labeling (TUNEL) kits (Millipore, USA). Sperm samples were put on poly-lysine-coated slides and dried at room temperature after being spun down and washed with PBS. In a staining process that followed the instructions from the manufacturer, the preparations were left at 37 °C for 60 minutes with a TUNEL mixture, and DNA fragments were marked. DAB was passed through chromogen and alcohol series after counter staining and coated with entellan. The Nikon Eclipse 80i light photomicroscope was used to count and study 200 sperm cells in each preparation. The X100 lens was used to take pictures of them.

Statistical Analysis

Statistical analyses of the study were performed with SPSS version 26.0 analysis program. Normality distribution of the groups was evaluated with Shapiro-Wilk Test. When it was found that all of the data were not normally distributed, the data were expressed as median±IQR. Differences of data between groups were evaluated with Kruskall-Wallis Test, which is a non-parametric test. Individual group differences were evaluated with post-hoc analysis Bonferroni Test. The level of significance was set at $p \leq 0,05$.

RESULTS

This study was done with sperms from people who asked for a semen analysis. According to WHO 2020 criteria, the study included samples from people with normal sperm. The effect on sperm parameters of adding different amounts of Mito-TEMPO to solutions used for cryopreservation was studied. When Mito-TEMPO was used instead of pre-cryopreservation, the percentage of cells with normal shape didn't change. It was found that the number of viable sperm cells was much lower after cryopreservation than before ($p<0.01$). When the groups' viability after cryopreservation was compared, the Mito-TEMPO group was found to have kept its viability much better than the other groups ($p<0.01$) (Figure 1).

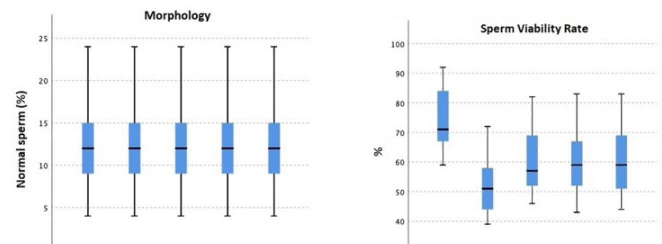

Figure 1. Pre-cryopreservation and post-cryopreservation morphology and viability values of the related groups.

When comparing the motility of all groups before and after cryopreservation, it was found that all groups had less motility after cryopreservation ($p<0.05$). When the groups were compared after cryopreservation, the highest motility loss was found in the group which was not added Mito-TEMPO ($p<0.05$). There was no significant difference between the group that got 1 µM Mito-TEMPO and the group that didn't get any, but the groups that got 10 µM and 50 µM Mito-TEMPO still moved around. The group to which 50 µM Mito-TEMPO was added had the most sperm that could move ($p<0.05$) (Figure 2).

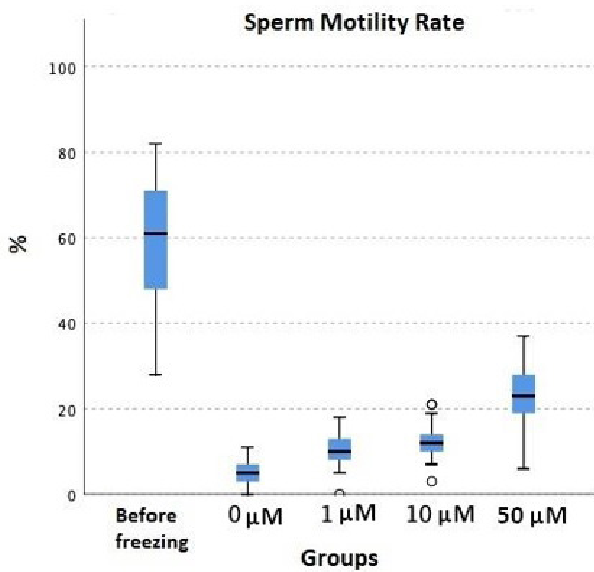

Figure 2. Pre-cryopreservation and post-cryopreservation motility values of the related groups.

When the groups were compared in terms of chromatin condensation, the rate of decondensed sperm was highest in the group that was not added Mito-TEMPO as a result of aniline blue staining ($p<0.01$) (Figure 3). When the group that was not added Mito-TEMPO and the groups that were added 1 µM and 10 µM Mito-TEMPO were compared, although fewer decondensed sperms were found in the group that was added 10 µM Mito-TEMPO, no statistical significance was found. The group closest to the chromatin condensation rate of sperm before cryopreservation was the group that was added 50 µM Mito-TEMPO ($p<0.05$) (Figure 4).

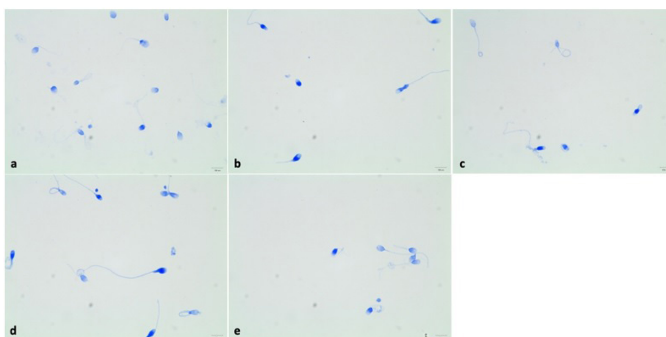

Figure 3. Pre-cryopreservation group (a), 0 µM Mito-TEMPO group (b), 1 µM Mito-TEMPO group (c), 10 µM Mito-TEMPO group (d), 50 µM Mito-TEMPO group, (d) 1000X, Scale bar: 10 µm. Condensed sperms are seen to be lightly stained with acidic aniline blue in pictures a, b, c, d and e; decondensed sperms are seen to be darkly stained with aniline blue in pictures a, b, c, d and e.

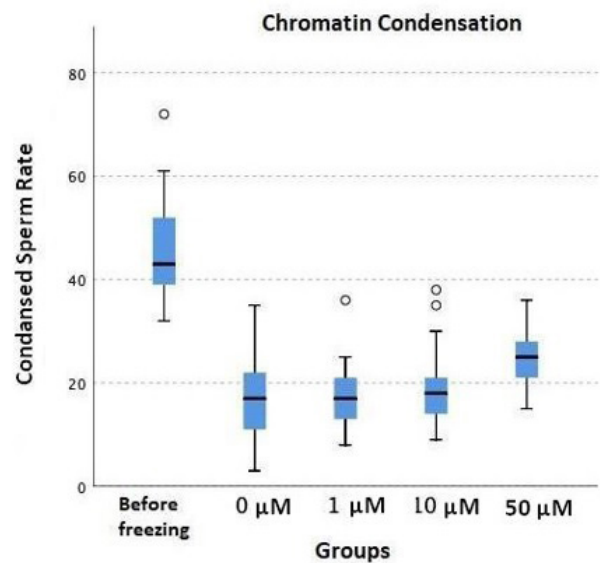

Figure 4. Pre-cryopreservation and post-cryopreservation condensed sperm values of the related groups.

When the sperm cells were compared in terms of apoptosis as a result of TUNEL staining (Figure 5), the apoptosis rate in Mito-TEMPO added groups (1µM -10µM -50µM) was statistically significantly lower than the group that was not added Mito-TEMPO ($p<0.05$) (Figure 6).

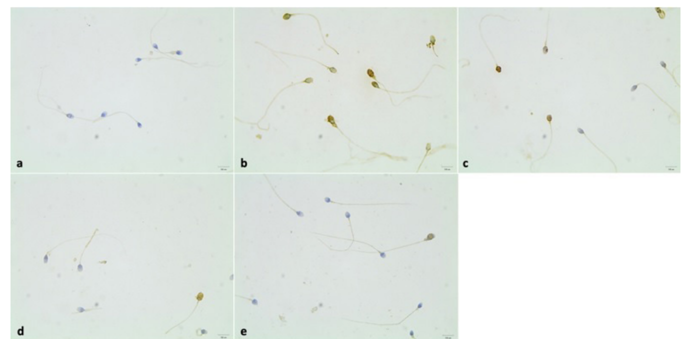

Figure 5. Pre-cryopreservation group (a), 0 µM Mito-TEMPO group (b), 1 µM Mito-TEMPO group (c), 10 µM Mito-TEMPO group (d), 50 µM Mito-TEMPO group, (d) 1000X, Scale bar: 10 µm. Apoptotic sperms are seen to be brown stained with TUNEL in pictures a, b, c, d and e; non-apoptotic sperms are seen to be blue stained with TUNEL in pictures a, b, c, d and e.

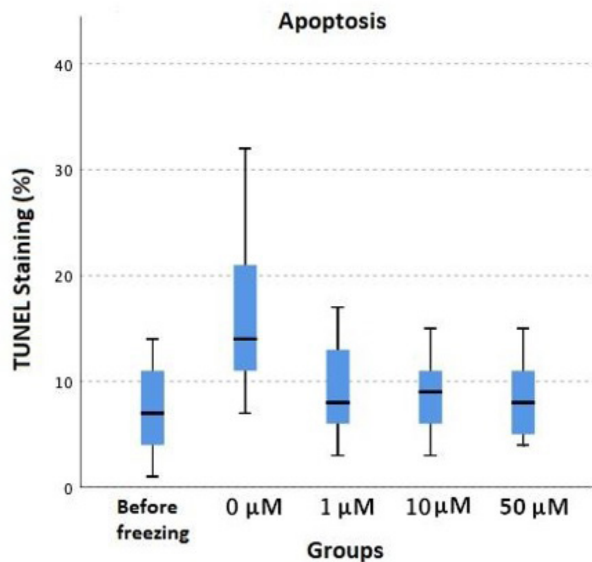

Figure 6. Pre-cryopreservation and post-cryopreservation apoptotic sperm values of the related groups.

DISCUSSION

Due to the small number of studies on how antioxidants that target mitochondria affect the freezing of sperm, this study looked at the role of different Mito-TEMPO concentrations in the freezing of human sperm. After freezing, the effects of Mito-TEMPO on the movement, shape, viability, chromatin integrity, and changes that look like apoptosis were studied. Viability and mitochondrial activity were found to decrease in sperms after freezing, and overall sperm motility was found to decrease in groups. Mito-TEMPO, on the other hand, significantly kept sperm mitochondrial activity and reduced the number of damaged mitochondria during incubation. This is likely because Mito-TEMPO goes straight to mitochondria and removes superoxide anions from mitochondria. Mito-TEMPO did not change the percentage of cells with normal shape in any of the groups. Mito-TEMPO may not have had any effect on the shape of normal and abnormal sperm because it was happened at the stage of spermatogenesis. The results of the morphology test agree with studies that say antioxidants don't change the shape of sperm in any way (10,14,15).

Cryopreservation is a key technique that is often used to keep men's ability to have children (5) and to store sperm in a stable way for a long time. Chemotherapy, radiotherapy, and surgery are all good ways to keep fertilization going before infertility interventions. Even though semen cryopreservation has made great strides in recent years, the damage caused by freezing still happens, and the quality of sperms decreases during the freezing-thawing process (16) During the cryopreservation process, oxidative stress is caused by an imbalance between ROS production and antioxidant mechanisms. Sperm cells are especially vulnerable to oxidative stress (8). When sperm is frozen, apoptotic pathways are also turned on, and ROS concentrations go up. When the amount of ROS in the body goes up, it damages the DNA of sperm and makes it harder for them to fertilize eggs. During cryopreservation, ROS are made, which changes the sperm's mitochondrial membrane potential (6). Cryopreservation changes how sperm membrane lipids are made, how long they live, and how they move. It also damages DNA in humans. Cryopreservation has a big effect on how well sperm move (17). Cryoprotectant solutions are used to lessen the stress that freezing causes. But at high concentrations, cryoprotectants are very bad for cells (4). So, cryopreservation methods need to be improved to make freezing protocol more effective (18). A lot of studies have shown that adding antioxidants to the process of freezing sperm can improve the quality of sperm that has been frozen and thawed in different ways (19–21). But there are still not enough good antioxidants (16).

Sperm endogenous antioxidants naturally neutralize ROS; however, the amount of these antioxidants decreases since seminal plasma is removed during the freezing-thawing process. For this reason, freezing medium containing supplement exogenous antioxidants is an effective method to overcome the negative effects of ROS (22,23).

While some antioxidants improve sperm functions, some may be insufficient. Antioxidants like Vitamin C, Vitamin E, catalase, quercetin, pentoxifylline, genstein, biotin, resveratrol, honey, and L-carnitine are commonly used (2).

Researchers have recently been interested in new types of antioxidants that target the mitochondria because they have many uses, work well, and don't harm the body. They have become possible ways to keep sperm from getting damaged by the stress of being frozen (24). Mitochondria is an important organelle for cell energy metabolism that modulates redox mechanism, cell development and death (9). Spermatozoa get energy for their metabolism from mitochondria through ATP synthase and oxidative phosphorylation. But they are sensitive to changes in temperature and the amount of reactive oxygen species (ROS). This makes it hard for ATP to move and lowers the quality of sperm. Its imbalance in getting rid of and making free radicals causes oxidative shock, which damages DNA and causes the cell to die (10,25). Motility, plasma membrane function, acrosome integrity, and overall viability all go down after thawing. After thawing, mobility has been said to drop from 50.6% to 30.3%. But the process by which motility goes down hasn't been fully explained yet (2).

Mito-TEMPO is an antioxidant that works on mitochondria and is a powerful ROS scavenger (26). This chemical is made by putting together tempo and triphenylphosphonium (TPP+). Tempo acts like superoxide dismutase, but while dismutase is a superoxide in the catalytic cycle, TPP is a cation that moves from the cell membrane to the cell interior (25).

This combination makes a compound that works well to remove superoxide from the mitochondria. Mito-TEMPO is also expected to keep the quality of human spermatozoa that have been frozen and then thawed (27,28). Weidinger et al. found that Mito TEMPO decreased the expression of nitric oxide synthase in the liver, as well as markers of liver and kidney damage (aspartate aminotransferase and alanine aminotransferase) (urea and creatinine) (27). Mito-Tempo is a good mitochondria-targeted antioxidant. Because it has a positive charge, it can build up 500 times in the mitochondrial matrix. It has a targeted antioxidant effect by stopping or slowing the production of mitochondrial free oxygen radicals and lipid peroxidation (24). Studies have also shown that Mito-TEMPO protects against diseases like heart damage (29), kidney damage (30), liver damage (9), Alzheimer's disease (31), sepsis models (32), Parkinson's disease (33), diabetes (34), ischemic brain disease (35), testicular toxicity (36), gastrointestinal system (37), and spinal cord damage (38).

Mito-TEMPO has been shown to improve mitochondrial function in porcine oocyte (39) and human spermatozoa (16) via decreasing oxidative stress. Seok et al. investigated the effects of different concentrations of Mito-TEMPO (0, 0,5, 5, 50 and 500 μM) on the motility of frozen-thawed porcine sperms. They proved that Mito-TEMPO addition had a beneficial effect on the motility of pig sperms (40). In another study, Kumar et al. added 50 μM Mito-TEMPO and 50 μM acetovanillone to freezing solution of buffalo sperm. When they examined semen samples in terms of progressive motility, plasma membrane integrity, lipid peroxidation, total antioxidant capacity, mitochondrial membrane potential and ROS, they reported that their separate or combined use affected the results positively (8).

In a study they conducted, Esmaeilkhanian et al. examined the efficiency of Mito-TEMPO (0, 1, 10,100 and 1000 μM) on post-thawing goat sperm quality. After thawing, they evaluated sperm mitochondria membrane potential, viability, apoptotic like changes and ROS concentration and they found that Mito-TEMPO (10 and 100 μM) improved sperm viability and reduced apoptotic like changes and ROS concentration when compared with other groups (22).

In a study they conducted on ram sperm, Zarei found that using Mito-TEMPO (0, 0.5, 5, 50 and 500 μM) improved thawed sperm motility parameters, membrane functionality, abnormal morphology, mitochondrial activity, acrosome integrity, DNA fragmentation, ROS concentration, viability and apoptotic like changes. According to the results, the efficiency of 5 and 50 μM Mito-TEMPO was found to be higher when compared with the other groups. In addition, apoptotic like changes were found to be lower in groups that were given lipid peroxidation and ROS concentration 5 and 50 μM Mito-TEMPO (10).

In a study they conducted on Verasfer variegatus, Zidni et al. froze sperms with different concentrations of Mito-TEMPO (0, 25, 50, 75, 100, 125, 150, 175 and 200 μM) and evaluated the post-thawing quality of sperms. When compared with control groups, antioxidant supplementation in sperm was found to be more effective in increasing post-thawing motility, maintaining cellular survival rates and preventing the increase in DNA damage that occurs in sperms during storing (7).

Zhang et al. found that in human sperm cryopreservation, Mito-TEMPO concentrations between 0 and 50 Mm showed significant improvement in post-thawing sperm motility, viability, membrane activity and mitochondrial membrane potential. They stated that adding Mito-TEMPO (10 and 100 μM) to cryopreservation solution improved sperm membrane integrity, mitochondrial membrane potential and chromatin integrity. They also found that Mito-TEMPO decreased the formation of oxidative stress and prevented mitochondria and DNA damage during cryopreservation (24).

In a study they conducted, Lu et al. stored semen samples by freezing them with diluents including different concentrations (0.0, 0.5, 5, 50 and 500 μM) of Mito-TEMPO. Sperm motility, viability, membrane integrity, mitochondrial membrane potential and antioxidant activities were measured. The results showed that adding Mito-TEMPO (5–50 μM) significantly increased post-thawing sperm motility, viability, membrane integrity and mitochondrial membrane potential. In the meantime, antioxidant enzyme activities increased and MDA content decreased in the group supplemented with Mito-TEMPO (16).

Asadzadeh et al. looked at the effects of Mito-TEMPO on ram sperm quality and fertility potential during the freezing-thawing process. They found that adding 5 μM and 50 μM Mito-TEMPO to semen samples led to higher post-thawing sperm motility, acrosome integrity, and viability, as well as lower lipid peroxidation and late apoptotic-like changes (25).

CONCLUSION

Using Mito-TEMPO improved deteriorated human sperm parameters during the thawing process. The damage that occurs during cryopreservation as a result of Mito-TEMPO effect can be prevented. Therefore, it can be said that adding Mito-TEMPO in diluent while thawing is an effective method in improving post-thawing sperm quality. In addition, more research is needed to investigate whether sperm fertilization, embryo implantation and pregnancy are affected. Further studies may help to clarify the mechanisms underlying protective role of Mito-TEMPO.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Analysis of the Relationship Between Serum Zinc Values and Allergic Rhinitis Parameters in Patients with Allergic Rhinitis

Alerjik Rinitli Hastalarda Serum Çinko Değerleri ile Alerjik Rinit Parametreleri Arasındaki İlişkinin Analizi

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Abstract

Background/Aim: Zinc is an significant trace element that acts an active part in the immune system and is associated with allergic inflammatory diseases. In the literature, various results have been shown in studies on the coexistence of serum zinc level and allergic rhinitis. The objective of this research was to analyze the intercourse among serum zinc levels and whole blood parameters in allergic rhinitis (AR).

Method: In this research, patients were appraised in consistency with the instruction for "Allergic Rhinitis and Its Effect on Asthma". Serum total Ig E, C-reactive protein grades in the AR group and leukocyte, neutrophil, eosinophil and lymphocyte counts, neutrophil/lymphocyte, eosinophil/lymphocyte, platelet/lymphocyte ratio and serum zinc values in whole-blood parameters of all cases were analyzed and contrasted between the AR and control groups.

Findings: Neutrophil and eosinophil counts and eosinophil/lymphocyte ratio were presented as significantly superior in the AR category than in the control category ($p<0.05$). Serum zinc values in the AR category were remarkably underneath than those in the control category ($p<0.05$). In the univariate model, it was noticed that the eosinophil and zinc values had a significant-independent differential effect in distinguishing the AR and control category patients ($p< 0.05$).

Conclusion: Serum zinc level, neutrophil and eosinophil count, eosinophil/lymphocyte ratio in the blood are practical biochemical indicators that can be used to recognition, treatment, together with following-up of cases with allergic rhinitis. It is assumed that with the support of zinc deficiency, important clinical benefits can be achieved in anti-allergic treatment.

Keywords: Rhinitis, allergic, trace elements, skin tests

Özet

Giriş/Amaç: Çinko, bağışıklık sisteminde aktif rol oynayan, alerjik inflamatuar hastalıklarla ilişkili önemli bir eser elementtir. Literatürde serum çinko düzeyi ile alerjik rinit hastalığının birlikteliği konusunda yapılan çalışmalarda farklı sonuçlar gösterilmiştir. Bu çalışmanın amacı, alerjik rinitte serum çinko seviyeleri ile tam kan değerleri arasındaki ilişkiyi araştırmaktır.

Yöntem: Alerjik rinit tanısı alan erişkin yüz otuz iki kişi hasta ve yüz otuz altı kişi sağlıklı kontrol grubu olarak incelenmiştir. Hastalar "Alerjik Rinit ve Astım Üzerindeki Etkisi" kılavuzuna göre değerlendirilmiştir. Hasta grubunda serum total IgE, C reaktif protein düzeyleri ile tüm olguların tam kan parametrelerinde lökosit, nötrofil, eozinofil ve lenfosit sayıları, nötrofil lenfosit, eozinofil lenfosit, platelet lenfosit oranı ve serum çinko değerleri incelenmiş, hasta ve kontrol grubu arasında karşılaştırılmıştır.

Bulgular: Hasta grubunda nötrofil ve eozinofil sayısı, eozinofil lenfosit oranı kontrol grubundan kayda değer ölçüde daha yüksek bulunmuştur ($p<0.05$). Hasta grubunda serum çinko değeri kontrol grubundan anlamlı olarak daha az tespit edilmiştir ($p<0.05$). Tek değişkenli modelde vaka ve kontrol grubu hastalarını ayırmada eozinofil, çinko değerinin anlamlı-bağımsız ayırıcı etkisi olduğu gözlenmiştir ($p< 0.05$).

Sonuç: Çinko, alerjik rinitte inflamasyonda rol alan önemli bir eser elementtir. Serum çinko düzeyi, kanda nötrofil ve eozinofil sayısı, eozinofil lenfosit oranı alerjik rinit hastalarında tanı, tedavi ve takipte kullanılabilecek pratik biyokimyasal göstergelerdir. Çinko eksikliğinin desteklenmesi ile anti alerjik tedavide önemli klinik faydalar sağlanılabileceği düşünülmektedir.

Türkçe Anahtar Kelimeler : Rinit, alerjik, eser elementler, deri testleri

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INTRODUCTION

Allergic rhinitis (AR) is an important global health problem. AR is one of the common complaints for applying to the otolaryngology (ENT) outpatient clinic. The mean prevalence of AR is about 18.1% (1%-54.5%) in the general population (1, 2).

It is known that systemic inflammation takes place in addition to nasal inflammation in AR pathogenesis. It is characterized by local collection of inflammatory cells such as; T-lymphocyte, mast cells, eosinophils, basophils, besides neutrophils in blood and tissue (3). AR results from immunoglobulin-E (IgE)-mediated reactions to inhaled allergens. It is a common chronic disease worldwide (4).

AR has a negative effect on daily activity, school performance, and academic achievement. It is also known to reduce the quality of life by causing a loss of workforce and sleep disorders (5).

The main symptoms of AR are rhinorrhea, nasal itching, sneezing, and nasal congestion. Rhinorrhea is usually profuse and serous in nature. Paroxysmal sneezing attacks are the most characteristic symptoms of AR and are accompanied by nasal itching and irritation (6). In the Allergic Rhinitis and its Impact on Asthma (ARIA) guideline, AR is classes as intermittent or persistent and mild or moderate/severe. Determination is bottomed on the practical records together with physical examination. In cases who have uncontrollable rhinitis or long-term symptoms despite medications, skin prick tests to identify the allergen or the presence of specific IgE antibodies against the allergen should be examined (4).

IgE has a main act in type I hypersensitivity, which reflects the sensitivity of mast cells by allergen-specific IgE antibodies bound to their high-affinity receptors (FcεRI) (7). Zinc (Zn) has numerous physiological functions. It is an prominent trace element that performs an significant act in the immune tract. Zn influences many perspectives of immune task, including thymic improvement and the actions of immune cells. Zn is also related in several steps of FceRI-induced mast cell activation required for degranulation and cytokine production. It has been indicated to impede the production and mRNA expression of inflammatory cytokines. Zn is known to task as an antioxidant and stabilize cell membranes. It has been stated that Zn has an important function in allergic inflammation (8, 9). This relationship among reduced Zn levels and asthma and atopic dermatitis has been more clearly demonstrated (10, 11).

Measurement of complete blood count parameters in AR patients is cost-effective and easy to perform. Recently, there has been a tendency to use eosinophil, lymphocyte, and neutrophil ratios instead of numerical values. Neutrophil/lymphocyte ratio (NLR), eosinophil/lymphocyte ratio (ELR), and platelet/lymphocyte ratio (PLR) can be computed easily (12).

Few studies have been directed on the role of mark elements in AR disease together with they have reported contradictory outcomes. Since different results have been reported in various studies, further studies are needed. In the textes, the number of studies on the relationship among serum total IgE and Zn levels is very scarce, and there is no study on the correlation between NLR, ELR and PLR, and Zn levels.

This research aims to investigate the intercourse among serum Zn and serum total IgE grades, blood neutrophil, eosinophil and lymphocyte counts, NLR, ELR, and PLR in AR.

MATERIAL and METHODS

Study Design

Between January 2021 and September 2022, 132 patients who asked to the outpatient clinic of the Faculty of Medicine Hospital, Department of Otorhinolaryngology were contained in the investigation. Cases over the age of 18 who were diagnosed with AR and presented to the ENT outpatient clinic with objections of itching in the nose and palate, nasal congestion, nasal discharge and sneezing, whose symptoms lasted more than four weeks, and more often than four days a week and did not receive any medical treatment, were evaluated. Errors were determined according to ARIA guidelines.

In the AR group, skin prick test results, C-reactive protein (CRP) levels, serum total IgE and blood parameters of leukocyte, neutrophil, eosinophil and lymphocyte counts, NLR, ELR, PLR and serum Zn values were recorded. NLR, ELR and PLR were calculated by dividing neutrophils, eosinophils and platelets by the percentage of lymphocytes in the complete blood count analysis.

One hundred thirty-six people of similar age groups and genders who did not have AR symptoms and who were received to the medical institution due to other complaints were joined in the research as the control group.

Patients in the pediatric age group (<18) and who were recently (less than 4 weeks ago) diagnosed with AR, with a history of nasal surgery, nasal polyps, oncological diagnosis, and pregnant cases were excepted from the research.

The investigation protocol was managed in pursuance with the ethical principles in the Declaration of Helsinki and was approved by the institutional ethics committee (approval date/number 19.10.2022/10-02). The authors declared that they followed the protocols used in the study centers regarding the publication of patient data.

Zinc Levels

Serum Zn levels were measured with the brand commercial kit (Archem Diagnostics (İstanbul, TURKEY)) using colorimetric method defined in the literature. (13).

Statistical analysis

Mean, standard deviation, median minimum, maximum, frequency and ratio values were used as descriptive statistics. The distribution of variables was analyzed with the Kolmogorov-Smirnov test. The Mann-Whitney u test (in analysis of quantitative independent data), the Chi-square test (in analysis of qualitative independent data) were used. ROC curve was used to investigate the effect level and cut-off value. The effect level was analyzed with univariate and multivariate logistic regression. SPSS 28.0 (IBM Corp. Armonk NY) program was used in the analysis.

RESULTS

Patient characteristics

In the study, 132 cases with AR besides 136 control cases were evaluated. The average age was 35.5 ± 12.5 in the AR group, and 38.9 ± 14.8 in the control group. The female/male ratio was 91/41 in the AR, besides 91/45 in the control group. There was not any statistically meannig ($p > 0.05$) difference with the age and gender dispersion of the cases between the AR and control group.

The skin prick test involved positive control, negative control, tree mix, olea europeae (olive tree), cockroach, cat epithelium, weed mix, weed mix, mold mix II and mite mix allergens. Single allergen positivity was interpreted as monoallergic, and multiple allergen positivity was interpreted as polyallergic condition. The outcomes of the derm prick assays revealed 31 monoallergic and 35 polyallergic cases. The prick test was negatory in 66 cases.

With regard to the biochemistry parameters, platelet and lymphocyte values, NLR and PLR values did not differ statistically between the AR and control group ($p > 0.05$).

Neutrophil and eosinophil counts and ELR values in the AR category were statistically remarkably ($p < 0.05$) superior than the control category. Elevated serum total-IgE levels were described as > 100 kU/L. Serum total IgE was elevated in 37 patients and was within normal limits in 95 cases. The findings are represented in tables 1 and 2.

Effect of serum Zn levels on the possibility of allergic sensitization

Serum Zn values in the AR category were statistically remarkably lower than the control category ($p < 0.05$) (Table 2, Figure 1). In the univariate model, a statistically significant ($p < 0.05$) differential effect of neutrophil, eosinophil, ELR and Zn values in differentiating the cases in AR and control group was observed, while a significant-independent ($p < 0.05$) differential effect of eosinophil and Zn values was observed in the multivariate model (Table 3).

The mean serum Zn level was 81.6±14.3. There is a significant difference in serum Zn levels among the cases in the AR and control groups [The ROC analysis; Area under the curve 0.758 (0.701-0.816)], and the cut-off value was obtained as 72.6 [Area under the curve 0.694 (0.630-0.758)]. (sensitivity: 93.4%, positive prediction: 45.5%, specificity: 63.8%, and negative prediction :75.8%) (Table 4).

Relationship between serum zinc level and allergic sensitivity

Platelet, lymphocyte, eosinophil counts and ELR, PLR and CRP values didn't differ statistically between the groups with serum Zn levels < 72.6 and ≥ 72.6 ($p > 0.05$). Neutrophil counts and NLR were remarkably higher ($p < 0.05$) in the category with serum Zn level < 72.6 than in the group with ≥ 72.6. In the group with serum Zn level < 72.6, the derm prick test and serum total IgE positivity rate were statistically higher ($p < 0.05$) (Table 5).

DISCUSSION

Low serum Zn has been related with various chronic diseases. There are a few studies examining the association among Zn levels together with allergic diseases, like allergic asthma and atopic dermatitis (14). However, there are studies with different results to explain the relationship (15).

In various studies conducted in adult and pediatric age groups, AR patients and healthy control groups were compared and it was shown that serum Zn was significantly lower in the AR patient group (16-18). Conversely, there are also works demonstrations that there is no significant distinction (15).

In our research, serum Zn values were determined to be significantly under in the AR category compared to the control category.

It has been determined in various investigations that Zn has an significant role in allergic inflammatory diseases, and it has been manifested to play a act in distinct IgE-related cellular signaling cascades (19). It has been statemented that there is an imbalance in IgE-dependent immunological activities in the presence of low Zn (19). Seo et al. investigated the relationship between total IgE and serum zinc levels in AR patients and showed that serum zinc grades were low in cases with elevated serum total IgE levels, and there was a negative linear correlation between total IgE levels and zinc levels (20).

In our research, it was noticed that the total IgE level was significantly superior in the group with serum Zn level < 72.6 contrasted to the group with Zn level ≥ 72.6. Our findings promoted the investigations in the written works.

Neutrophilia is one of the systemic inflammatory markers and is associated with inflammatory diseases (21). Eosinophilic inflammation is a common feature in allergic diseases. The eosinophil count is used to state the intensity of allergic diseases. In their research, Li et al. statemented that the eosinophil count and serum total IgE level were higher in patients with AR (22).

NLR and ELR are simple biochemical parameters that can be easily performed in the detection of inflammatory and infectious illnesses (21, 23). Yenigün et al. showed that NLR and ELR values increased as a result of the systemic inflammatory reply in nasal polyposis disease (21). In their study on children with functional dyspepsia, an inflammatory disease, Savas et al. pointed out a statistically significant distinction in NLR values between the control and the AR group. Although there was numerical distinction in serum Zn levels among the groups, it was not statistically significant (24).

In a research by Kant et al., eosinophil counts and ELR quantities were noticed to be remarkably higher and NLR values lower in AR cases matched to the healthful ones (25). Göker et al. found higher NLR and PLR values in AR patients matched to the healthful group (26).

In our investigation, neutrophil and eosinophil levels were higher in the AR group. Serum Zn levels and ELR values were found to be effective in differentiating the AR and control groups. In multivariate logistic regression analysis, eosinophil count and Zn levels were found as significant independent discriminative variables. These results support that Zn levels and eosinophilia are parameters associated with AR.

This investigation has some limitations. It is uncertain whether a individual evaluation of serum Zn level exactly matches the actual serum Zn level in the population.

Since it was not checked whether there was an improvement in AR patients after Zn deficiency treatment, obvious information could not be acquired about the role of Zn deficiency in the intensity and treatment of the disease.

Knowledge on the act of serum total IgE as a marker in AR intensity and diagnosis is restricted and changeable. This issue should be addressed further.

Failure to look at the correlation of allergen-specific IgE with Zn level created a limitation in the specificity of the study.

CONCLUSION

Zn is a crucial trace element involved in the definition of the severity and therapy of chronic inflammatory diseases. The low serum Zn levels detected in AR patients in our study support the facts of other investigations in the literary texts. Morover, the association between neutrophilia, eosinophilia, high ELR, low Zn levels and AR indicates that a low Zn level might be associated with AR disease. Checking the serum Zn concentration is beneficial in the aftercare and therapy of AR disease, and we trust that it may be a useful strategy for advancing anti-allergy treatments.

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TABLES

Table 1. Demographic data of the entire cohort

		Min-Max	Median	Mean ± sd/ n (%)
Age		18.0 - 84.0	34.0	37.2 ± 13.8
Gender	Female			182 (67.9%)
	Male			86 (32.1%)
Platelet (x10³)		79.0 - 532.0	272.0	279.7 ± 68.8
Neutrophil (x10³)		0.97 - 9.00	3.89	4.13 ± 1.38
Lymphocyte (x10³)		0.78 - 42.50	2.22	2.56 ± 2.75
Eosinophil (x10³)		0.00 - 1.85	0.16	0.22 ± 0.21
NLR		0.58 - 6.81	1.70	1.88 ± 0.79
ELR		0.00 - 0.95	0.07	0.10 ± 0.10
PLR		1.0 - 475.2	119.9	125.1 ± 45.9
CRP		0.10 - 2.00	0.10	0.19 ± 0.25
Zinc		43.2 - 143.8	79.9	81.6 ± 14.3
Prick Test	Non			66 (50.0%)
	Mono			31 (23.5%)
	Poly			35 (26.5%)
Total IGE	High			37 (28.0%)
	Normal			95 (72.0%)
Rhinitis				132 (49.3%)
Healthy controls				136 (50.7%)

Table 2. Comparison of AR and control groups

		AR Group		Control Group		p
		Mean ± sd/ n(%)	Median	Mean ± sd/ n (%)	Median	
Age		35.5 ± 12.5	34.0	38.9 ± 14.8	35.5	0.080 ^{m}
Gender	Female	91 (68.9%)		91 (66.9%)		0.722 $^{X^2}$
	Male	41 (31.1%)		45 (33.1%)		
Platelet (x10³)		286.5 ± 63.9	276.5	273.0 ± 72.7	265.0	0.052 ^{m}
Neutrophil (x10³)		4.32 ± 1.35	4.10	3.94 ± 1.40	3.62	***0.013*** ^{m}
Lymphocyte (x10³)		2.67 ± 3.55	2.29	2.45 ± 1.63	2.19	0.363 ^{m}
Eosinophil (x10³)		0.26 ± 0.25	0.17	0.18 ± 0.15	0.15	***0.003*** ^{m}
NLR		1.92 ± 0.71	1.75	1.84 ± 0.87	1.60	0.102 ^{m}
ELR		0.11 ± 0.12	0.08	0.08 ± 0.07	0.06	***0.005*** ^{m}
PLR		124.2 ± 38.8	119.6	125.9 ± 52.0	120.1	0.530 ^{m}
Zinc		75.7 ± 12.8	74.1	87.4 ± 13.4	86.8	***<0.001*** ^{m}
^{m} Mann-Whitney u test / $^{X^2}$ Chi-Square test						

Table 3. Evaluation of the effectiveness of laboratory parameters with Univariate and Multivariate analysis

	Univariate Model			Multivariate Model		
	OR	%95 CI	p	OR	%95 CI	p
Neutrophil	1.22	1.02 - 1.46	***0.027***			
Eosinophil	10.76	2.33 - 49.63	***0.002***	11.21	2.14 - 58.65	***0.004***
ELR	58.22	2.42 - 1403.7	***0.012***			
Zinc	0.928	0.906 - 0.950	***<0.001***	0.927	0.904 - 0.950	***<0.001***
Logistic Regression (Forward LR)						

Table 4. Evaluation of the effectiveness of serum zinc level in separating AR and control groups by ROC analysis

	Area under the curve	%95 Confidence Interval	p
Zinc	0.758	0.701 - 0.816	***<0.001***
Zinc Cut Off Value 72.6	0.694	0.630 - 0.758	***<0.001***

		Control Group	AR Group		%
Zinc	< 72.6	60	9	Sensitivity	93.4%
	≥ 72.6	72	127	Positive Prediction	45.5%
				Specificity	63.8%
				Negative Prediction	87.0%

Table 5. Comparison of AR patients according to serum zinc ≥ 72.6 cut off value

		Zinc < 72.6		Zinc ≥ 72.6		p
		Mean ± sd/ n (%)	Median	Mean ± sd/ n (%)	Median	
Age		36.2 ± 14.2	33.0	37.6 ± 13.7	35.0	0.326 ^{m}
Gender	Female	55 (79.7%)		127 (63.8%)		***0.015*** $^{X^2}$
	Male	14 (20.3%)		72 (36.2%)		
Platelet (x10³)		286.3 ± 70.1	273.0	277.4 ± 68.3	271.0	0.333 ^{m}
Neutrophil (x10³)		4.60 ± 1.33	4.40	3.97 ± 1.37	3.69	***<0.001*** ^{m}
Lymphocyte (x10³)		2.25 ± 0.59	2.19	2.66 ± 3.16	2.25	0.341 ^{m}
Eosinophil (x10³)		0.24 ± 0.23	0.16	0.21 ± 0.21	0.16	0.892 ^{m}
NLR		2.19 ± 0.96	1.95	1.77 ± 0.69	1.60	***<0.001*** ^{m}
ELR		0.11 ± 0.10	0.07	0.09 ± 0.10	0.07	0.469 ^{m}
PLR		134.7 ± 53.9	126.0	121.7 ± 42.4	117.0	0.062 ^{m}
CRP		0.20 ± 0.30	0.10	0.18 ± 0.20	0.10	0.666 ^{m}
Zinc		65.5 ± 5.7	66.1	87.2 ± 12.0	85.1	
Prick Test	Non	20 (33.3%)		46 (63.9%)		***0.001*** $^{X^2}$
	Mono	22 (36.7%)		9 (12.5%)		
	Poly	18 (30.0%)		17 (23.6%)		
Total IgE	(+)	25 (41.7%)		12 (16.7%)		***0.001*** $^{X^2}$
	(-)	35 (58.3%)		60 (83.3%)		

FIGURES

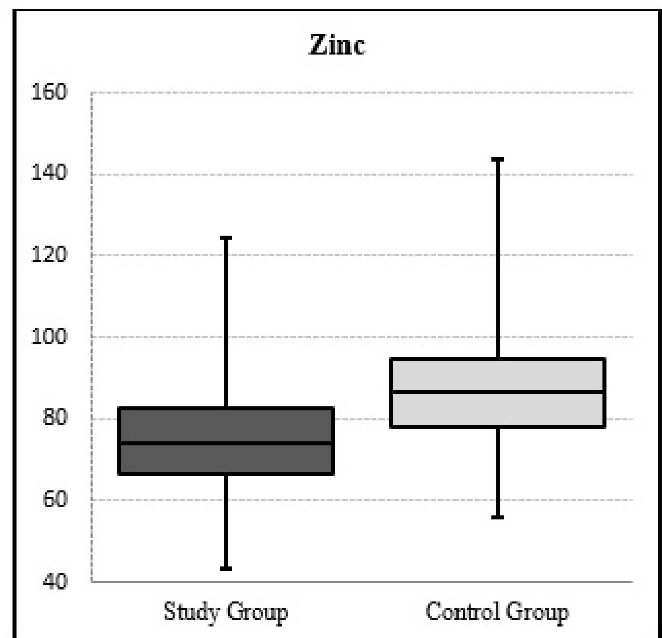

Figure 1. Evaluation of the effectiveness of serum zinc level in separating AR and control groups by ROC Cure