Original Article / Özgün Araştırma

The Effect of Niacin and Melatonin Supplementation on the Antioxidant System and Lipid Peroxidation in Exercised Rats

Egzersiz Yaptırılan Ratlarda Niasin ile Melatonin Takviyesinin Antioksidan Sistem ve Lipid Peroksidasyonu Üzerine Etkisi

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Abstract

Objective: The aim of this study was to examine the effect of niacin and melatonin supplementation on the antioxidant system and lipid peroxidation in exercised rats.
Materials and Methods: The rats were divided into four groups. Running exercise was done for ten days. Niacin and melatonin were given before exercise.
Results: The malondialdehyde levels of the melatonin group were lower than those of the other groups. Malondialdehyde levels of the niacin + melatonin group were higher than the control groups super oxide dismutase (SOD) levels of the melatonin group were higher than those of the other groups. SOD levels of the niacin + melatonin group were lower than those of the other groups. Catalase levels of the melatonin group were higher than those of the niacin groups. Glutathione peroxidase levels of the control group were lower than those of the other groups. Total oxidant levels of the control group were lower than those of the niacin groups. Total antioxidant level (TAS) levels of the control group were lower than those of the other groups. TAS levels of the niacin + melatonin group were lower than those of the niacin or melatonin groups. Melatonin levels of the melatonin groups were higher than those of the other groups.
Conclusion: Melatonin supplementation decreased malondialdehyde levels and increased antioxidant parameters. It can be said that it has a higher antioxidant effect compared to niacin. Co-administration of niacin and melatonin increased oxidation parameters and decreased SOD and catalase levels. These enzymes indicated that they were used in response to increased oxidant status.

Keywords
Exercise, melatonin, niacin, lipid peroxidation, antioxidant

Anahtar Kelimeler
Egzersiz, melatonin, niasin, lipid peroksidasyonu, antioksidan

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Öz

Amaç: Bu çalışmanın amacı, egzersiz yaptırılan ratlara verilen niasin ve melatonin desteğinin antioksidan sistem ve lipid peroksidasyonuna olan etkisini incelemektir.
Gereç ve Yöntemler: Ratlardan 4 grup oluşturuldu. On gün koşu egzersizi yaptırıldı. Egzersiz öncesi niasin ve melatonin verildi.
Bulgular: Melatonin grubunun malondialdehit düzeyleri, diğer gruplara göre daha düşüktü. Niasin + melatonin grubunun malondialdehit düzeyleri, kontrol grubuna göre daha yüksekti. Melatonin grubunun Süper oksit dismutaz (SOD) düzeyleri, diğer gruplara göre daha yüksekti. Niasin + melatonin grubunun SOD düzeyleri, diğer gruplara göre daha düşüktü. Melatonin grubunun katalaz düzeyleri, niasin gruplarına göre daha yüksekti. Kontrol grubunun glutatyon peroksidaz düzeyleri, diğer gruplara göre daha düşüktü. Kontrol grubunun total oksidan seviye düzeyleri, niasin gruplarına göre daha düşüktü. Kontrol grubunun total antioksidan seviye

(TAS) düzeyleri, diğer gruplara göre daha düşüktü. Niasin + melatonin grubunun TAS düzeyleri, niasin veya melatonin gruplarına göre daha düşüktü. Melatonin gruplarının melatonin düzeyleri diğer gruplara göre daha yüksekti.
Sonuç: Melatonin takviyesi, malondialdehit düzeylerini azalttı ve antioksidan parametreleri artırdı. Niasine göre daha yüksek bir antioksidan etkiye sahip olduğu söylenebilir. Niasin ve melatoninin birlikte uygulanması oksidasyon parametrelerini arttırdı ve SOD ve katalaz seviyelerini azalttı. Bu enzimler, artan oksidan durumuna yanıt olarak kullanıldıklarını gösterdi.

Introduction

In recent years, studies on exercise have reported that in addition to its beneficial effects on health, exercise also has oxidant effects depending on the type and intensity of the exercise (1,2). It is known that regular exercise has beneficial effects on health; however, its molecular mechanisms on peripheral tissues have not been examined adequately yet (1). Especially intense exercise and hypoxia are reported to have cumulative effect on stress (2). It is known that contracting skeletal muscles form free radicals and cellular structures are exposed to oxidative damage during long lasting intense exercise (3,4).

It is reported that melatonin detoxifies free radicals such as OH^- and H_2O_2 and therefore can prevent the harmful effects of these on biomolecules (5). Melatonin also protects the membrane against lipid peroxidation by attaching to the outer surface of the phospholipid layer of the cell membrane and contacting the radicals before the membrane (3).

There are contradictory studies about the application of melatonin before exercise; it has been shown that giving 1 mg/kg of melatonin to rats undergoing acute exercise reduces oxidative damage (4). On the other hand, in another study investigating the effects of melatonin in rats exposed to intense exercise, it has been shown that melatonin has no effect on total antioxidant status (TAS) and total oxidant status (TOS) (6).

The first substance in the synthesis of melatonin and niacin is tryptophan. Niacin is the precursor of NAD+/NADH and NADP+/NADPH that undertake important metabolic events in living cells. It has an active role in preventing many pathological processes with the effect of this metabolic cofactor (7). In studies conducted with niacin, it is reported to affect a large number of antioxidant enzymes; however, there are contradictory studies on the issue (8).

When studies conducted on oxidant state and antioxidant capacity with both melatonin and niacin were examined, no studies were found in which the effects of melatonin and niacin, which have their origins from a common, essential amino acid, were evaluated together. There are also some contradictory studies such as the insufficiency of their antioxidant capacity. In line with this information, the aim of this study was to investigate the effects of administering melatonin and niacin, which originate from the same amino acid, separately or together on lipid peroxidation and antioxidant capacity in the face of oxidative stress caused by exercise.

Materials and Methods

In this study, Procedures on animals were performed in Aydın Adnan Menderes University (ADU) Faculty of Medicine Experimental Animal Center. All analyzes were performed in ADU Central Laboratory. The ethics committee decision required for the study was obtained from ADU Animal Experiments Local Ethics Committee Center (Decision no: 64583101/2018/039, date: 23.03.2018). This study is the study of the doctoral thesis completed at ADU, Institute of Health Sciences, Department of (Medicine) Biochemistry. This study was supported by ADU Scientific Research Unit.

Procedures Applied on Rats

In the study, 40 rats weighing 380-465 grams were used. Rats were divided into four groups (n=10). Group 1= control group (only exercise was done), group 2= niacin group (exercise + niacin), group 3= melatonin group (exercise + melatonin), group 4= niacin + melatonin combo group (exercise + niacin + melatonin).

Rats exercise treadmill jogging at 15 min/day and 20 m/min speed without inclination for 10 days. Melatonin (Sigma-Aldrich) was given at a dose of 5 mg/kg intraperitoneally 30-45 minutes before exercise. Niacin (Sigma-Aldrich) was given orally at a dose of 360 mg/kg 30-45 minutes before exercise.

Approximately 7 mL of blood samples were collected by intracardiac, one day after the last exercise. Blood specimens taken were transferred to ethylenediaminetetra-acetic acid tubes for catalase study and to biochemistry tubes for other studies. Serum samples were used for Malondialdehyde (MDA), TOS, TAS, melatonin, super oxide dismutase (SOD) and glutathione peroxidase (GPX)measurment.

Biochemical Methods

Catalase, MDA, SOD, GPX, TOS, TAS and melatonin levels were determined by spectrophotometric methods. Shimadzu Uv-1700 (Japan) device was used for catalase. TOS and TAS analysis were performed with the Rel Assay (Turkey) kit. Melatonin measurment was performed with the Elabscience kit. Biotek Epoch (Canada) device was used for SOD, MDA, GPX, TOS, TAS and melatonin analysis.

Statistical Analysis

SPSS 22.0 Windows program was used to analyze the data. Results were given as mean ± standard deviation. The groups were compared One-Way ANOVA. As a post hoc test, Tukey test was used for analyzed parameters MDA, Catalase, TOS, TAS and melatonin, and Dunnett T3 test was used for SOD and GPX.

Results

The rats were weighed every day of the exercise. Along with exercise, weight loss was observed in groups.

The group melatonin (2.09±0.67 μmol/L) gave lower MDA concentrations in comparison to control (3.01±0.53 μmol/L), niacin (3.23±0.50 μmol/L) and niacin + melatonin (3.77±0.54 μmol/L) groups ($p<0.01$) (Figure 1). However, higher MDA levels were found in the group received niacin + melatonin (3.77±0.54 μmol/L) in comparison to control (3.01±0.53 μmol/L) and melatonin (2.09±0.67 μmol/L) groups ($p<0.001$).

SOD levels were higher in group melatonin (12.52±2.57 U/mL) in comparison to control (8.74±1.08 U/mL), niacin (8.37±1.01 U/mL) and niacin + melatonin (6.01±0.61 U/mL) groups, while low SOD levels were observed in the group niacin + melatonin (6.01±0.61 U/mL) in comparison to control (8.74±1.08 U/mL), niacin (8.37±1.01 U/mL) and melatonin (12.52±2.57 U/mL) groups ($p<0.01$) (Figure 2).

Catalase levels were higher in the group melatonin [11.47±2.19 k/gr hemoglobin (Hb)] in comparison to niacin (8.19±2.48 k/gr Hb) ($p<0.05$) and niacin

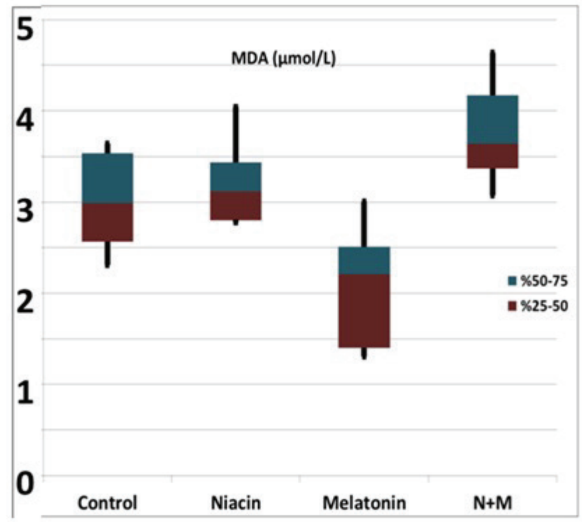

Figure 1. MDA levels of the groups
MDA: Malondialdehyde

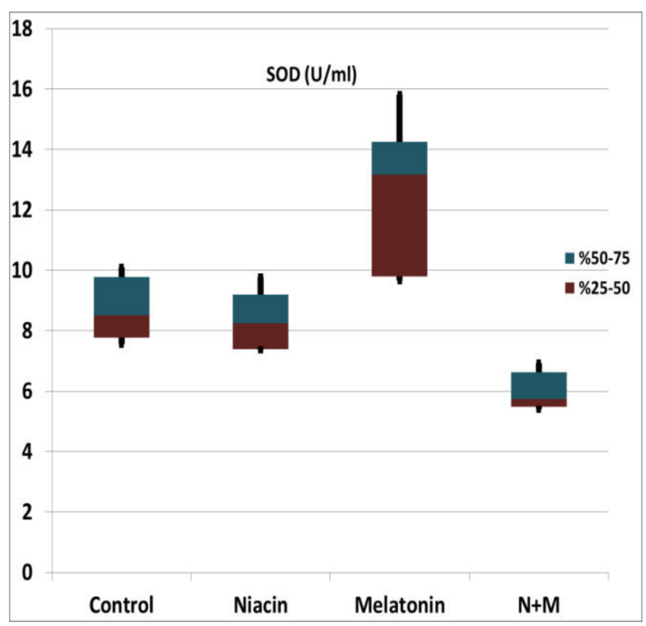

Figure 2. SOD levels of the groups
SOD: Super oxide dismutase

+ melatonin (6.95±1.95 k/gr Hb) ($p<0.001$) groups (Figure 3).

GPX levels were low in the control group (526.00±99.54) in comparison to melatonin (1029.92±249.34), niacin (1033.44±198.05) and niacin + melatonin (1152.33±313.19) combo groups ($p<0.001$) (Figure 4).

TOS levels were low in the control group (11.46±2.01 μmol H_2O_2 Equiv./L) in comparison to niacin (15.43±2.69 µmol H2O2 Equiv./L) and niacin + melatonin (14.89±2.99 µmol H2O2 Equiv./L) groups ($p<0.05$) (Figure 5).

Lower levels of TAS was found in the control group (797.61±44.2 µmol Trolox Equiv./L) in comparison to melatonin (1205.32±38.84 µmol Trolox Equiv./L), niacin (1188.83±54.27 µmol Trolox Equiv./L) and niacin + melatonin (1113.56±72.11 µmol Trolox Equiv./L) combo groups ($p<0.001$). Additionally,

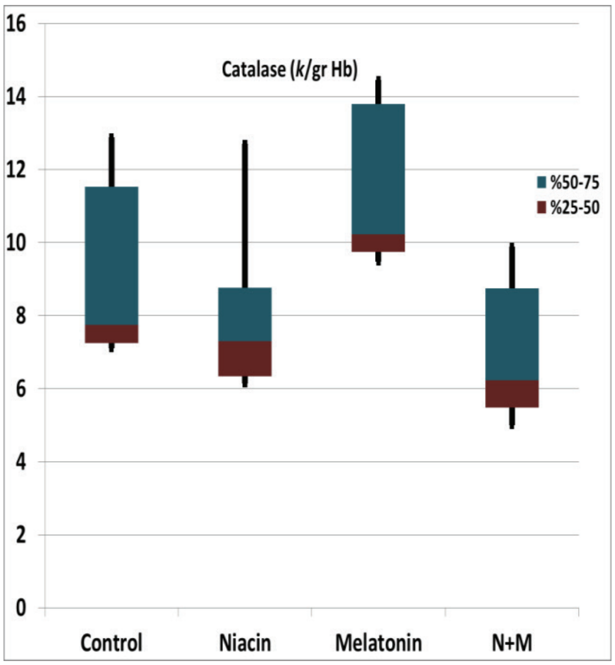

Figure 3. Catalase levels of the groups

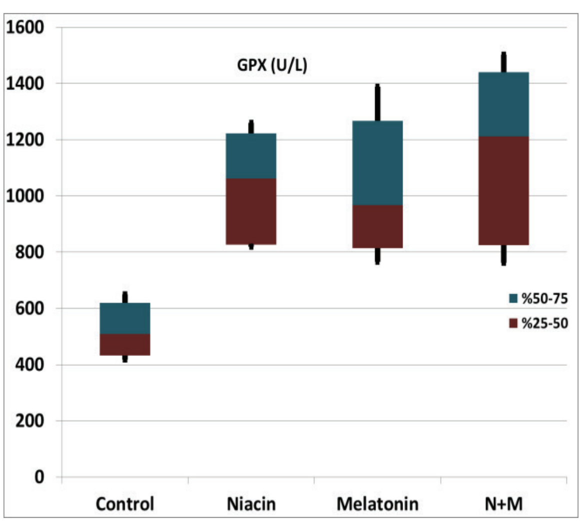

Figure 4. GPX levels of the groups
GPX: Glutathione peroxidase

niacin+melatonin group (1113.56±72.11 µmol Trolox Equiv./L) showed lower levels in comparison to melatonin (1205.32±38.84 µmol Trolox Equiv./L), niacin (1188.83±54.27 µmol Trolox Equiv./L) groups ($p<0.05$) (Figure 6).

Melatonin levels were higher in the groups received melatonin (189.09±39.85 pg/mL) and niacin

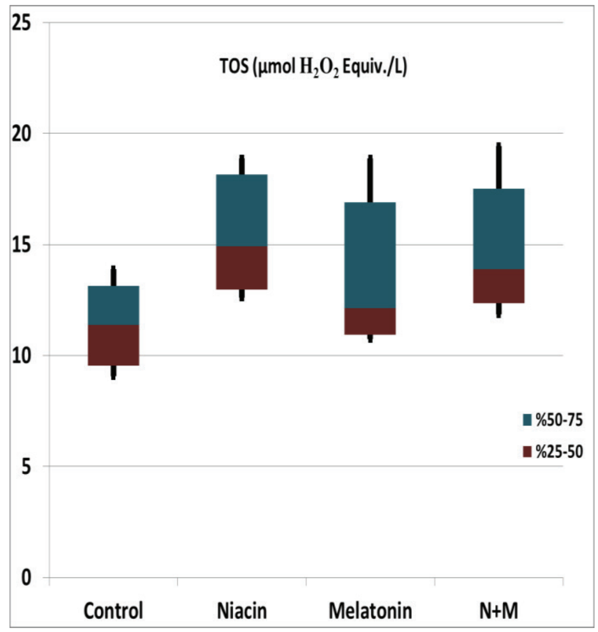

Figure 5. TOS levels of the groups
TOS: Total oxidant status

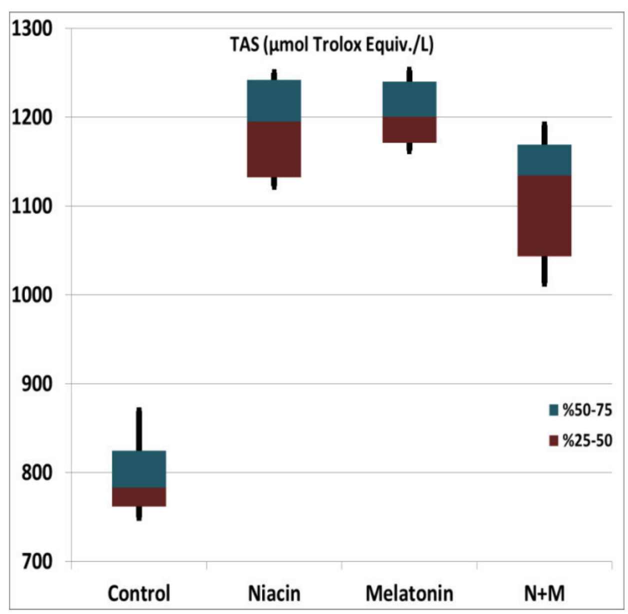

Figure 6. TAS levels of the groups
TAS: Total antioxidant level

+ melatonin (189.71±31.48 pg/mL) in comparison to control (135.72±26.81 pg/mL), niacin (142.80±24.80 pg/mL) groups ($p<0.05$) (Figure 7).

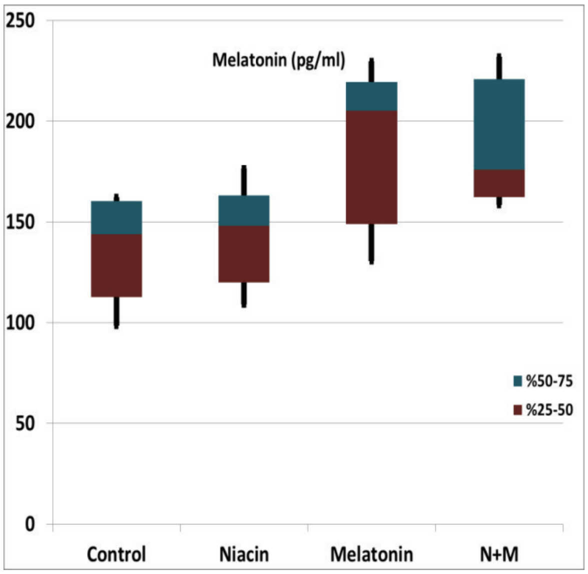

Figure 7. Melatonin levels of the groups

Discussion

It is known that moderate and intense exercise increases oxidant activity, is harmful for the organism and can threaten health. In our study, our aim was to examine the antioxidant effects of melatonin and niacin, which are reported to have antioxidant effect but the effects of which can change depending on the type of physical activity, separately and in combination on exercised rats.

Oxidative stress is known to play a role in a large number of physiological situations such as aging or exercise and in various diseases. Although regular mild exercise seems to be useful for health and oxidative stress, acute and tiring workouts of anaerobic and aerobic exercise may trigger a review of systems (ROS) over production (9). Though exercise is a source of stress that creates oxidative stress by increasing the production of free oxygen radicals, It is also known to affect antioxidant enzyme activity to develop resistance against oxidative stress (10).

In our study, rats were exposed to treadmill exercise with no elevation for 10 days. The control group was just exposed to exercise and the other three groups that exercised were given niacin, melatonin, niacin + melatonin, respectively.

The group that experienced the most significant weight loss during exercise was the group niacin+melatonin together. Malondialdehyde levels were higher in the group with niacin and melatonin together than in the other groups. MDA levels of the melatonin group were found to be lower than the other groups. In exercised rats, melatonin alone was found to decrease lipid peroxidation. MDA levels of the group that was given only niacin were found to be significantly higher than those of the group that was given only melatonin. It can be said that niacin is not as effective as melatonin in preventing lipid peroxidation.

It has been shown that long term exercise in adult men increases MDA levels and that melatonin significantly decreases MDA increase (11). In our study, similarly, MDA was lower in the group melatonin when compared with the other groups.

In another study which compared obese children and healthy subjects, oxidant-antioxidant state was examined in association with weight loss. Body mass index (BMI), fat mass and waist-hip ratio were found to decrease significantly, while MDA was found to increase significantly, when compared with the control group. MDA was correlated with whp, fat mass and BMI and antioxidant state was found to return to normal after six months of dietary restriction (12).

In a study in which 400 mg/kg niacin was given to rats and exposed to exercise, it was found that NAD content and energy consumption increased in skeletal muscle (13). In a study conducted on rats, it was found that melatonin application caused SOD levels to be higher (14). In this study, SOD levels of the group melatonin was found to be higher when compared with the other groups.

In a study in which the effect of aerobic exercise on lipid metabolism was investigated in rats with dietary hyperlipidemia, it was reported that malondialdehyde levels increased, while SOD levels decreased and SOD levels increased after MDA levels decreased (15). In our study, weight loss, decrease in SOD levels and increase in malondialdehyde levels were observed especially in the group niacin + melatonin.

Catalase is one of the antioxidant enzymes that catalyse the decomposition of H_2O_2 formed by SOD into molecular oxygen and water (16). In a study which examined the effect of melatonin against oxidative stress induced by exercise in healthy individuals, no significant change was observed in catalase level (17). In our study, significant change was not found in the group melatonin when compared with the control group. In our study, weight loss and catalase were found to decrease in the group niacin+melatonin together when compared with the group melatonin.

In a study in which melatonin was made in athletes, pre and post-exercise GPX levels of the control group and the groups which received melatonin were examined. Post-exercise levels were found to be lower in both groups when compared with the pre-exercise. In the comparison between groups, the group that was given melatonin was found to be higher when compared with the control group (18).

Studies conducted on the antioxidant effect of niacin have been conducted on different living beings and cell cultures. Niacin has been found to decrease ROS production in hepatocyte cell cultures (19).

In our study, when compared with the control group, GPX levels of the other three groups were found to be higher. In all of the groups that received niacin, GPX level was found to be significantly high, unlike SOD, catalase and MDA levels.

When the analysed TOS levels were examined in our study, the levels of the two groups given niacin, which had more weight loss when compared with the other two groups, were found to be significantly high when compared with the control group. The kit we used in our study to determine TAS gives results about the total antioxidant state with TEAC method. TAS levels of the groups which were given melatonin and niacin were found to be significantly high when compared with the control group. These results show that niacin and melatonin show antioxidant separately and together.

In our study, when serum melatonin levels were examined, it was found that the two groups which received melatonin had significantly higher serum melatonin levels than the two groups which did not receive melatonin. This result showed that antioxidant and oxidant parameters were evaluated in suitable melatonin concentrations.

Melatonin increases the volume of muscle spindles and decreases ROS production (20). The indole ring of melatonin prevents free radical damage, it has also been shown to be very effective in increasing physical performance (21). The effect of giving melatonin on physical performance is still a disputable issue. In their meta analysis, Lopez-Flores et al. (22) reported that the effect of giving melatonin could differ depending on the type of physical activity. There are a large number of studies reporting that giving exogenous melatonin in experimental animal studies has antioxidant effects and repairs muscle damage in rats (23-25). In our study, similar to the results of the studies above, giving melatonin to rats increased antioxidant activity.

Conclusion

As a result, it can be said that the administration of exogenous melatonin to rats increases antioxidant activity and also shows higher antioxidant activity than niacin. It can be said that the administration of exogenous niacin to rats increases antioxidant activity (GPX, TAS). It can be said that the administration of exogenous niacin and melatonin combination to rats did not create a synergistic effect on antioxidant parameters.

Ethics

Ethics Committee Approval: The present cohort study was designed as a survey and was approved by the Local Ethical Committee for Animal Experiments of Aydın Adnan Menderes University (decision no: 64583101/2018/039, date: 23.03.2018).

Informed Consent: Experimental study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.K., Concept: A.K., A.B.K., Design: A.K., A.B.K., Data Collection or Processing: A.K., A.B.K., Analysis or Interpretation: A.K., Literature Search: A.K., A.B.K., Writing: A.K., A.B.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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