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Effects of gonadotropin releasing hormone antagonist (GNRHant) and oral progestin-primed protocol on oocyte count over the punctured follicle number in consecutive two cycles: A comparative study

Gonadotropin salgılatıcı hormon antagonisti (GNRHant) ve oral progestin astarlı protokolün ardışık iki siklusta delinmiş folikül sayısı üzerinden oosit sayısı üzerine etkileri: Karşılaştırmalı bir çalışma

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Abstract

Objective: Controlled ovarian hyperstimulation plays a critical role in in vitro fertilization (IVF) success. However, premature luteinization and variations in oocyte yield can impact IVF outcomes. This comparative study aims to investigate the effects of gonadotropin releasing hormone antagonist (GNRHant) and oral progestin-primed protocol on the oocyte count over the punctured follicle number in the same patient group undergoing consecutive IVF cycles.

Materials and Methods: Forty-nine patients undergoing IVF were enrolled in this comparative study. Each participant underwent two consecutive IVF cycles. In the first cycle, GNRHant protocol was used. In the second cycle, the OPP protocol was used. The number of punctured follicles and oocytes retrieved was recorded and compared between the two cycles for each patient.

Results: The ratio of oocyte count per punctured follicle number was higher in the OPP group compared to the GNRHant group, without clinical significance (p>0.05). In the OPP, the ratio of oocytes retrieved over the punctured follicle number was 0.90±0.28; in the GNRHant group, it was recorded as 0.94±0.36, and the differences between the ratios were statistically insignificant.

Conclusion: Oocyte yield is a critical determinant of IVF success, and it can be influenced by various factors, including premature luteinization and follicular development. The use of GNRHant and OPP is known to prevent premature luteinization and improve follicular synchronization. This study demonstrates that neither of the protocols is superior in the success of oocyte retrieval over the punctured follicle count. Further research with larger sample sizes and randomized controlled trials is warranted to validate these results, and optimize clinical application of this combined protocol in IVF treatments.

Keywords: Infertility, gonadotropin releasing hormone antagonist, controlled ovarian hyperstimulation, oral progestin-primed protocol

PRECIS: In a comparative study of consecutive in vitro fertilization cycles, the oocyte yield relative to punctured follicles was similar between gonadotropin releasing hormone antagonist and oral progestin-primed protocols, showing no significant clinical advantage.

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Öz

Amaç: Kontrollü ovaryan hiperstimülasyon, in vitro fertilizasyon (IVF) başarısında kritik bir rol oynamaktadır. Bununla birlikte, erken luteinizasyon ve oosit verimindeki farklılıklar IVF sonuçlarını etkileyebilir. Bu karşılaştırmalı çalışma, ardışık IVF siklusları uygulanan aynı hasta grubunda Gonadotropin salgılatıcı hormon antagonisti (GNRHant) ve oral progestinle uyarılan protokolün delinmiş folikül sayısı üzerinden oosit sayısı üzerindeki etkilerini araştırmayı amaçlamaktadır.

Gereç ve Yöntemler: IVF uygulanan 49 hasta bu karşılaştırmalı çalışmaya dahil edilmiştir. Her katılımcıya iki ardışık IVF siklusu uygulanmıştır. İlk döngüde GNRHant protokolü kullanılmıştır. İkinci döngüde ise oral progestinle hazırlanan (OPH) protokol kullanılmıştır. Delinen foliküllerin ve alınan oositlerin sayısı kaydedilmiş ve her hasta için iki döngü arasında karşılaştırılmıştır.

Bulgular: Delinmiş folikül sayısına göre oosit sayısı OPH grubunda GNRHant'a kıyasla daha yüksek olup klinik olarak anlamlı değildir (p>0,05). OPH'de, alınan oositlerin delinen folikül sayısına oranı 0,90±0,28 iken GNRHant grubunda bu oran 0,94±0,36 olarak kaydedilmiştir ve korelasyon istatistiksel olarak anlamsızdır.

Sonuç: Oosit verimi IVF başarısının kritik bir belirleyicisidir ve erken luteinizasyon ve foliküler gelişim dahil olmak üzere çeşitli faktörlerden etkilenebilir. GNRHant ve OPH kullanımının erken luteinizasyonu önlediği ve foliküler senkronizasyonu iyileştirdiği bilinmektedir. Bu çalışma, her iki protokolün de delinmiş folikül sayısı üzerinden oosit alımının başarısı konusunda birbirlerine üstün olmadığını göstermektedir. Bu sonuçları doğrulamak ve IVF tedavilerinde bu kombine protokolün klinik uygulamasını optimize etmek için daha büyük örneklem boyutları ve randomize kontrollü çalışmalarla daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: İnfertilite, gonadotropin salgılatıcı hormon antagonisti, kontrollü ovaryan hiperstimülasyon, oral progestin destekli protokol

Introduction

Assisted reproductive technologies (ART) have undergone significant advancements in recent years, offering a multitude of protocols to enhance the outcomes of in vitro fertilization (IVF). Among the various protocols employed, the gonadotropin releasing hormone antagonist (GNRHant) and oral progestin-primed (OPP) protocols have gained prominence for their efficacy in controlled ovarian hyperstimulation (COH). Understanding the nuanced impact of these protocols on crucial parameters such as oocyte count and punctured follicle numbers holds paramount importance for optimizing ART success.

GnRH plays a pivotal role in regulating the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), influencing the ovarian follicular development[1]. GnRH antagonists have been widely employed to prevent premature ovulation, thereby allowing precise control over the follicular maturation process during COH[2]. Conversely, the OPP protocol involves the administration of oral progestin prior to gonadotropin stimulation, exerting a modulatory effect on the endogenous gonadotropin release and ultimately impacting ovarian response[3].

While both GNRHant and OPP protocols have individually demonstrated success in promoting oocyte maturation and retrieval, there exists a paucity of studies directly comparing their effects on consecutive IVF cycles. This lacuna in the literature prompts the need for a comprehensive comparative analysis, aiming to elucidate potential differences in oocyte yield and follicular puncture outcomes between the two protocols across consecutive cycles.

This manuscript presents a thorough investigation into the effects of the GNRHant and OPP protocols on oocyte count relative to the punctured follicle number in consecutive IVF cycles. By critically examining and comparing these two widely utilized protocols, we aim to contribute valuable insights that can inform clinical decision-making, refine ART practices, and ultimately enhance the overall success rates of assisted reproduction.

Materials and Methods

Study Design

This study utilized a retrospective comparative design, examining data sourced from patient records at the Acıbadem Hospital IVF Unit for fertility treatment. The research adhered to ethical guidelines and received approval from the Acıbadem University Ethical Committee, with the assigned (approval number: 2022-04/137 no: 10.03.2023). The study adhered to ethical standards outlined in the Declaration of Helsinki. Patient confidentiality and privacy were strictly maintained throughout the study.

Study Participants

The study included a cohort of women undergoing consecutive IVF cycles at Acıbadem Hospital IVF Unit. Inclusion criteria comprised women aged 20-45 years, with a diagnosis of infertility and undergoing IVF treatment using the GNRHant for the first cycle and OPP protocol for the consecutive COH.

Treatment Protocols

GNRHant protocol

On the third day of the menstrual cycle, controlled ovarian stimulation was initiated by administering daily doses of r-FSH (300 IU follitropin alpha, Gonal-F, Serono, Geneva, Switzerland, or 16 mcg follitropin delta, Rekovelle®, Ferring, Saint-Prex, Switzerland). Upon visualizing at least one follicle ≥14 mm, patients received a subcutaneous injection of 0.25 mg GnRH antagonist (GnRHa, Cetrotide®; Merck KGaA, Darmstadt, Germany). When three or more follicles reached a mean diameter of ≥17 mm, and adequate serum estradiol levels were observed, administration of r-FSH and GnRH antagonist

were discontinued. Final follicular maturation was triggered by subcutaneous administration of recombinant human chorionic gonadotropin (r-hCG, Ovidrel®, Merck KGaA, Darmstadt, Germany). In cases where a patient was at risk of developing ovarian hyperstimulation syndrome (OHSS), a GnRH agonist (0.2 mg triptorelin acetate, Gonapeptyl daily®, Ferring GmbH, Kiel, Germany) was administered subcutaneously, instead of r-hCG. Oocyte retrieval was performed 37 hours after r-hCG or GnRH analog administration through transvaginal follicular aspiration guided by ultrasound, during which the patient underwent sedation. Metaphase II oocytes were selected for intracytoplasmic sperm injection (ICSI).

OPP Protocol

On the third day of the menstrual cycle, controlled ovarian stimulation was initiated by administering daily doses of r-FSH (300 IU follitropin alpha, Gonal-F, Serono, Geneva, Switzerland, or 16 mcg follitropin delta, Rekovelle®, Ferring, Saint-Prex, Switzerland). The r-FSH dosage was adjusted based on follicular development, monitored through ultrasound scans.

In the oral progesterone-primed group, starting from the third day of the cycle, patients received oral doses of medroxyprogesterone acetate (10 mg/day, Tarlusal®, Deva, Türkiye) until the trigger day. Oocyte retrieval was performed 37 hours after r-hCG or GnRH analog administration through transvaginal follicular aspiration guided by ultrasound, during which the patient underwent sedation. Metaphase II oocytes were selected for ICSI.

Data Collection and Analysis

Relevant clinical and demographic data, including age, body mass index (BMI), and baseline hormonal levels, were extracted from patient records. The primary outcomes, namely oocyte count and punctured follicle number, were analyzed using appropriate statistical methods (e.g., t-tests, chi-square tests). Subgroup analyses were conducted to explore potential variations in outcomes across different age groups and other relevant factors.

Limitations

Potential limitations, such as the retrospective nature of the study and the influence of confounding variables, were acknowledged.By employing these rigorous methodologies, the study aimed to provide robust insights into the comparative effects of the GNRHant and OPP protocols on oocyte count and punctured follicle numbers across consecutive IVF cycles.

Results

The study was conducted at Acıbadem Hospital between June 2019 and July 2023, involving a total of 49 women whose ages ranged from 20 to 45 years, with a mean age of 35.76±5.21.

The number of cycles among the participants ranged from 2 to 5, with a mean of 2.51±0.74. Among the participants, 20.4% (n=10) were found to have low ovarian reserve as the cause of infertility, while 16.3% (n=8) had genetic anomalies, 8.2% (n=4) had advanced maternal age, 22.4% (n=11) had male factor infertility, and 32.7% (n=16) were diagnosed with unexplained infertility.

The BMI measurements ranged from 16.2 to 37.9 kg/m^2, with a mean of 23.94±4.07 kg/m^2.

Concerning pregnancy, 67.3% (n=33) did not conceive, 24.5% (n=12) conceived once, and 8.1% (n=4) conceived two or more times.

Concerning the antral follicle count (AFC) on day 3 (D3), participants on GNRHant had a range of 2 to 32, with a mean of 10.61±6.34, while they had a range of 2 to 30 on the OPP protocol, with a mean of 10.22±5.94. No statistically significant difference was observed in AFC measurements between both protocols for the same patient cohort (p>0.05). The expected number of oocytes on GNRHant protocol ranged from 1 to 26, with a mean of 7.46±5.43, while on OPP, it ranged from 1 to 24, with a mean of 8.23±5.70. No statistically significant difference was found in expected oocyte count between the two protocols (p>0.05). The number of oocytes retrieved for GNRHant protocol ranged from 1 to 26, with a mean of 7.18±5.81, and for OPP, it ranged from 1 to 31, with a mean of 7.88±6.49 without clinical significance (p>0.05). The oocyte/AFC ratio on GNRHant protocol ranged from 0.25 to 2, with a mean of 0.94±0.36. On OPP, it ranged from 0.25 to 1.57, with a mean of 0.90±0.28, without any clinical significance, although there was a slight increase observed on GNRHant protocol compared to OPP (p>0.05). (Figure 1) Regarding endometrial thickness, GNRHant protocol had a range of 6.2 to 16.3, with a mean of 11.16±2.81, while OPP had a range of 5 to 16.6, with a mean of 9.48±9.30. GNRHant had a statistically

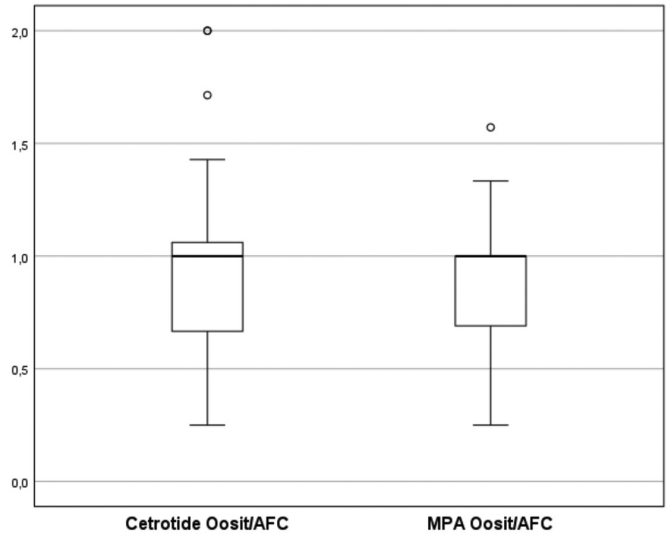

Figure 1. Distribution of retrieved occyte over the expected oocyte count for GNRHant and OPP protocols

GNRHant: Gonadotropin releasing hormone antagonist, OPP: Oral progestin-primed, AFC: Antral follicle count

significant thicker endometrium compared to OPP, with a mean difference of 1.78±2.29 mm (p=0.001; p<0.01) (Figure 2). On the trigger day, Estradiol (E2) levels for GNRHant ranged from 98 to 7499 pg/mL, with a mean of 1578.83±1347.91 pg/mL, while for OPP, the levels ranged from 297 to 8977 pg/mL, with a mean of 1555.07±1487.34 pg/mL. No statistically significant difference was found in trigger day E2 levels between both protocols (p>0.05). The number of Day 3 embryos obtained from GNRHant ranged from 0 to 14, with a mean of 3.14±3.39, and for OPP, it ranged from 0 to 19, with a mean of 4.27±3.75. GNRHant had a statistically significantly lower number of Day 3 embryos compared to OPP, with a mean difference of 1.31±3.03 (p=0.003; p<0.01) (Table 1). The number of Day 5 embryos obtained from GNRHant users ranged from 0 to 6, with a mean of 0.66±1.58, and for OPP, it ranged from 0 to 8, with a mean of 1.23±2.25. No statistically significant difference was found in the number of Day 5 embryos obtained between both protocols (p>0.05) (Table 2).

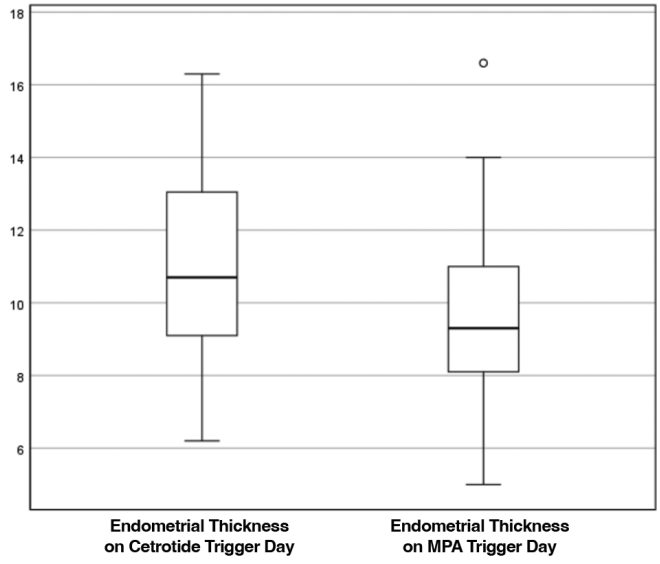

Figure 2. Distribution of endomerial thickness on the trigger day of GNRHant and OPP protocols

GNRHant: Gonadotropin releasing hormone antagonist, OPP: Oral progestin-primed, MPA: Medroxyprogesterone acetate

Table 1. Descriptive characterictics

Age	Mean ± SD Median (min-max)	36.76±5.21 36 (20-45)
BMI	Mean ± SD Median (min-max)	23.94±4.07 22.8 (16.2-37.9)
Number of IVF cycles	Mean ± SD Median (min-max)	2.51±0.74 2 (2-5)
Cause of inftiliy	Diminished ovarian reserve Genetic cause Advanced maternal age Male infertility Unexplained	10 (20.4) 8 (16.3) 4 (8.2) 11 (22.4) 16 (32.7)

IVF: In vitro fertilization, BMI: Body mass index, SD: Standard deviation

Statistical Analysis

SPSS 27 (Statistical Package for the Social Sciences) software was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used for data evaluation. Normal distribution of quantitative data was tested using the Kolmogorov-Smirnov test, Shapiro-Wilk test, and graphical analysis. The dependent samples t-test was used for intra-group comparisons of quantitative variables showing normal distribution. The Wilcoxon signed-ranks test was used for intra-group comparisons of quantitative variables not exhibiting normal distribution. Statistical significance was accepted as p<0.05.

The results presented here provide a comprehensive overview of the outcomes associated with the GNRHant and OPP protocols in consecutive IVF cycles. The observed differences/similarities in oocyte yield, punctured follicle numbers, and age-specific responses contribute valuable insights for clinicians and researchers in the field of assisted reproductive technologies.

Discussion

The present study aimed to compare the effects of GNRHant protocol and the OPP protocol on oocyte count and the number of punctured follicles over two consecutive IVF cycles. Our findings revealed notable differences between the two protocols in terms of oocyte yield and ovarian response; shedding light on their distinct mechanisms of action and potential clinical implications.

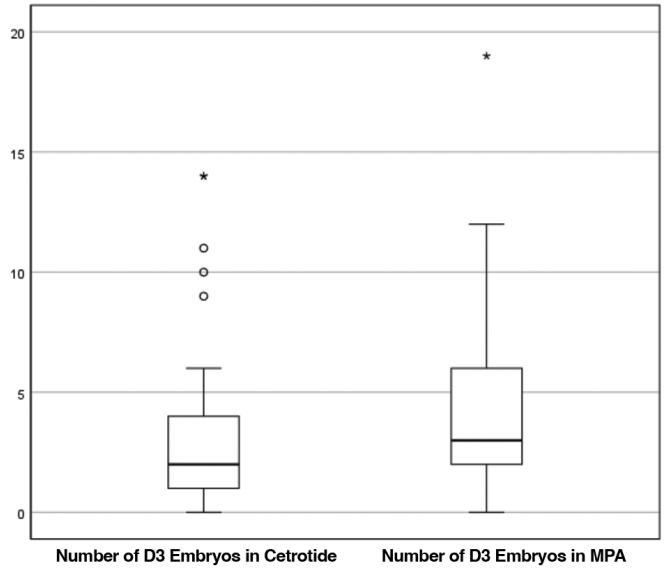

Figure 3. Distribution of D3 embryo number of GNRHant and OPP protocols

GNRHant: Gonadotropin releasing hormone antagonist, OPP: Oral progestin-primed, MPA: Medroxyprogesterone acetate

Table 2. GNRHant ve OPP evaluation

		GNRHant	OPP	Difference	test value; p
D3 AFC	Mean ± SD Median (min-max)	10.61±6.3 9 (2-32)	10.22±5.94 9 (2-30)	0.39±3.22 0 (-9-7)	t= 0.843 [a]**0.403**
Expected ooycte count	Mean ± SD Median (min-max)	7.46±5.43 6 (1-26)	8.23±5.70 7 (1-24)	-0.54±3.46 0 (-10-7)	Z=-0.895 [b]**0.371**
Retrieved oocyte count	Mean ± SD Median (min-max)	7.18±5.81 6 (1-26)	7.88±6.49 6.5 (1-31)	-0.58±3.76 0 (-11-8)	Z=-1.097 [b]**0.273**
Oocyte/AFC	Mean ± SD Median (min-max)	0.94±0.36 1 (0.25-2.0)	0.90±0.28 1 (0.25-1.57)	0.04±0.41 0 (-0.5-1.67)	Z=-0.257 [b]**0.798**
Endometrial thickness	Mean ± SD Median (min-max)	11.16±2.81 10.7 (6.2-16.3)	9.48±9.30 2.3 (5-16.6)	1.78±2.29 1.45 (-2.4-7.8)	t=5.151 [a]**0.001****
Trigger day E2	Mean ± SD Median (min-max)	1578.83±1347.91 1289.5 (98-7499)	1555.07±148.34 1272 (297-8977)	-37.34±734.50 -13 (-1662-1252)	Z=-0.168 [b]**0:866**
Number of D3 embryo	Mean ± SD Median (min-max)	3.14±3.39 2 (0-14)	4.27±3.75 3 (0-19)	-1.31±3.03 -1 (-8-7)	Z=-2.953 [b]**0.003****
Number of D5 embryo	Mean ± SD Median (min-max)	0.66±1.58 0 (0-6)	1.23±2.25 0 (0-8)	-0.50±1.98 0 (-7-6)	Z=-1.743 [b]**0.081**

[a]: Paired samples test, [b]: Wicoxon signed ranks test **: p<0.01, D3: Day 3, AFC: Antral follicle count, GNRHant: Gonadotropin releasing hormone antagonist, OPP: Oral progestin-primed, SD: Standard deviation

Moreover, while our study focused on oocyte count and punctured follicle numbers as key outcome measures, future research should investigate the impact of these protocols on live birth rates, pregnancy outcomes, and long-term maternal and neonatal health. A comprehensive understanding of the clinical implications of different ovarian stimulation protocols is essential for optimizing ART success rates and improving patient outcomes.

The study cohort consisted of 49 women with a diverse range of infertility etiologies, including low ovarian reserve, genetic anomalies, advanced maternal age, male factor infertility, and unexplained infertility. Notably, the mean age of participants was 35.76 years, reflecting a typical demographic profile for IVF treatment. The use of diverse patient populations enhances the generalizability of the study findings and underscores the relevance of the results in clinical practice.

In comparing the GNRHant and OPP protocols, the study observed no significant differences in the number of oocytes retrieved, expected oocyte count, or punctured follicle numbers between the two protocols. These findings align with previous studies suggesting comparable ovarian response and oocyte yield between GNRHant and progestin-primed protocols[4]. However, it's important to note, that while the oocyte count may not significantly differ between protocols, the nuances in ovarian response, as indicated by punctured follicle numbers, offer additional insights into the efficacy of ovarian stimulation regimens.

In the randomised controlled trial of Jabarpour et.al recently showed that viable embryo count is higher in OPP group compared to GNRHant group which is aligning with the outcome our study underscoring the significantly decrease in obtained day 3 embryos of the patients when they have undergone GNRHant protocole compared OPP[5].

Generally, rates of live births, ongoing pregnancies, clinical pregnancies, and pregnancy loss per embryo transfer are found to be similar between PPOS and GnRH analogue cycles. However, in certain analyses, particularly when examining patients with polycystic ovarian syndrome, PPOS may show a significantly higher clinical pregnancy rate per embryo transfer [Yildiz et al.[6]; Ata et al.[7]; Ata and Kalafat[8]].

This comparative study provides valuable insights into the differential effects of the GNRHant and OPP protocols on oocyte count and punctured follicle numbers in consecutive IVF cycles. The findings underscore the importance of protocol selection based on individual patient characteristics and treatment goals. By addressing these key points, our study contributes to the growing body of knowledge surrounding ovarian stimulation protocols, guiding clinicians in optimizing treatment strategies and ultimately improving ART success rates.

Conclusion

In conclusion, our comparative study meticulously examined the effects of GNRHant and OPP protocols on oocyte count and punctured follicle numbers, and their embryologic outcomes across consecutive IVF cycles. Even the slight discrepancies among the protocols emphasize the protocol-specific impact on the quantity of retrieved oocytes, underscoring the importance of tailored protocol selection in optimizing IVF outcomes. Further prospective investigations and randomized controlled trials are warranted to validate these findings, refine treatment

algorithms, and ultimately improve the success rates and safety profiles of IVF procedures. As we advance in understanding the intricacies of ovarian stimulation, the path to optimizing fertility treatments becomes clearer, offering successful outcomes with tailored solutions to individuals seeking assisted reproduction.

Ethics

Ethics Committee Approval: The research adhered to ethical guidelines and received approval from the Acıbadem University Ethical Committee, with the assigned (approval number: 2022-04/137 no: 10.03.2023). The study adhered to ethical standards outlined in the Declaration of Helsinki.
Informed Consent: Retrospective study.

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Footnotes

Authorship Contributions

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