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# Clinical correlates of treatment adherence and insight in patients with schizophrenia

# Şizofreni hastalarında tedavi uyumu ve içgörünün değerlendirilmesi

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Abstract

Aim: The aim of this study was to assess clinical correlates of the treatment adherence and insight in patients with schizophrenia.

Methods: That cross-sectional study included 229 outpatients with schizophrenia who were admitted to the Psychiatry Outpatient Clinic of Health Sciences University Dışkapı Yıldırım Beyazıt Training and Research Hospital. All participants were administered a socio-demographic form, Morisky Medication Adherence Questionnaire, Schedule for Assessing the three components of insight, Brief Psychiatric Rating Scale, Positive and Negative Symptoms Rating Scale, Calgary Depression Scale for Schizophrenia, and Global Assessment of Functioning Scale. Collected data were analyzed with descriptive statistics, Pearson Correlation Analysis, and logistic regression analysis.

Results: Poor treatment adherence was associated with male gender, lower insight level, more severe psychotic symptoms, and lower functionality level. The insight score was negatively correlated with the severity of psychotic symptoms, duration of the disorder, and mean antipsychotic dose; but positively correlated with advanced age of onset, and higher functionality level. The logistic regression analysis revealed that functionality level was more predictive on poor medication adherence.

Conclusion: Poor treatment adherence and lower insight level were closely associated with more severe clinical symptoms and lower functionality level. It was noteworthy that adherence and insight levels both showed a high predictivity for wellbeing of the patients. Therefore, psychotherapeutic interventions should be implemented to increase treatment adherence and insight in schizophrenia even if the psychotic symptoms show resistance. Further research is needed to clarify clinical associations of the treatment adherence and insight level in patients with schizophrenia.

Keywords: treatment adherence, schizophrenia, insight

Öz

Amaç: Bu çalışmanın amacı şizofreni hastalarında tedaviye uyumu ve içgörü ile ilişkili klinik değişkenleri değerlendirmektir.

Yöntemler: Kesitsel nitelikteki bu çalışmaya Sağlık Bilimleri Üniversitesi Dışkapı Yıldırım Beyazıt Eğitim ve Araştırma Hastanesi Psikiyatri Polikliniği'ne başvuran 229 şizofreni hastası dahil edildi. Tüm katılımcılara sosyodemografik veri formu, Morisky Tedaviye Uyum Ölçeği, İçgörünün Üç Bileşenini Değerlendirme Ölçeği, Kısa Psikiyatrik Değerlendirme Ölçeği, Pozitif ve Negatif Belirtileri Değerlendirme Ölçeği, Calgary Şizofrenide Depresyon Ölçeği ve İşlevselliğin Genel Değerlendirilmesi Ölçeği uygulanmıştır. İstatiksel analizde tanımlayıcı analizler, Pearson Korelasyon Analizi ve lojistik regresyon analizi kullanıldı.

Bulgular: Şizofrenide düşük tedavi uyumu bulunması erkek cinsiyet, düşük içgörü düzeyi, yüksek psikotik belirti şiddeti ve düşük işlevsellik düzeyi ile ilişkili bulundu. İçgörü düzeyi ise psikotik belirti şiddeti, hastalığın süresi ve ortalama antipsikotik ilaç dozu ile negatif korelasyon gösterirken; geç başlangıç yaşı ve yüksek işlevsellik düzeyi ile pozitif korelasyon gösterdi. Lojistik regresyon analizinde ise işlevsellik düzeyinin düşük tedavi uyumunu öngörmede daha etkili olduğu belirlendi.

Sonuç: Düşük tedavi uyumu ve içgörü düzeyi yüksek klinik belirti şiddetiyle ve düşük işlevsellik düzeyiyle güçlü korelasyon gösterdi. Tedavi uyumu ve içgörü düzeyi hastaların iyilik durumu üzerinde önemli prediktif faktörler olarak saptandı. Bu nedenle psikotik belirtiler dirençli olsa dahi şizofreni hastalarında tedavi uyumu ve içgörüyü arttırmaya yönelik psikososyal tedaviler uygulanmalıdır. Şizofreni hastalarında tedavi uyumu ve içgörü düzeyi ile ilişkili klinik değişkenlerin daha iyi anlaşılması için gelecekte yapılacak çalışmalar önemli olacaktır.

Anahtar Kelimeler: tedavi uyumu, şizofreni, içgörü

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## Introduction

Schizophrenia is a chronic disorder and shows poor clinical outcomes. One of the main reasons affecting on the clinical course of schizophrenia is poor treatment adherence. Many patients with schizophrenia drop out the medication in less than one year, and full adherence to treatment in patients with schizophrenia is not common [1]. Treatment adherence is defined as regular use of the drugs and compatibility with implemented suggestions of the healthcare staff [2]. Good treatment adherence is a necessity for the patients with schizophrenia to maintain improvement, however, there is a high rate of poor medication adherence in schizophrenia patients. Poor medication adherence in schizophrenia is associated with more relapses, increased number of hospitalizations, and poor clinical course [3].

Clinical course of schizophrenia is affected by several clinical factors, such as characteristics of the disorder, comorbid psychiatric disorders, and medication adherence [4]. Insight level is also a predictive factor on clinical course of schizophrenia [5]. Insight is basically defined as awareness of the disorder and signs of exacerbation. The insight concept also includes ability to interpret clinical symptoms as pathological and acceptance of the treatment [6,7]. A recent study assessed the impact of psychoeducation on knowledge level about the disorder and insight in patients with schizophrenia. That study revealed that psychoeducation did not have a significant impact on insight but provided a significant improvement on clinical symptoms [8]. Even if better insight level is not improved by the clinical interventions, the interventions might improve clinical outcomes in schizophrenia patients [8, 9].

Poor treatment adherence and low insight level often show a good clinical association in schizophrenia; however, poor medication adherence can be found in patients with good clinical insight [9,10]. Besides that, some patients with lower insight can be provided an adequate antipsychotic treatment [11]. Taken together, there are some differences between clinical associations of the medication adherence and insight. As both are major predictive factors on clinical course of schizophrenia, it is important to clarify clinical associations of the treatment adherence and insight in schizophrenia. A better understanding on the clinical associations of those two variables might improve clinical outcomes and therefore it was aimed to assess correlates of the treatment adherence and insight in patients with schizophrenia.

## Material and methods

This study was carried out in Dışkapı Yıldırım Beyazıt Training and Research Hospital. The patients aged between 18 and 60 with the diagnosis of schizophrenia according to Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) who applied to the outpatient Psychiatry Clinic of the Hospital between December 2017 and June 2018 were enrolled into the study [12]. The patients with remission and partial remission were included and patients who are not in remission/ partial remission (n=21) were not got involved. In addition, those with organic brain disorders (n=8), neurodevelopmental disorders (n=2), and alcohol or substance use disorders (n=5) were excluded. Therefore, 229 patients were included as participants. The study was carried out in compatibility to the Declaration of Helsinki. Written informed consents were obtained from all of the participants, and Ethical Commission of the Dışkapı Training and Research Hospital approved the study (06.11.2017-4210).

Instruments

Brief Psychiatric Rating Scale (BPRS): The BPRS is used to evaluate general symptom severity in patients with schizophrenia. It was developed by Overall and Gorham in 1961 [13]. It is an 18-item Likert scale, and all items are scored between 1 (absent) and 7 (severe) points. Its Turkish validity and reliability were made by Soykan et al. in 1989 [14].

Scale for the Assessment of Negative Symptoms (SANS): The SANS measures negative symptom severity such as anhedonia, avolition, and the items are rated from 0 to 5. It was developed by Andreasen in 1990 and its Turkish validity and reliability study was conducted by Erkoç et al. in 1991 [15,16].

Scale for the Assessment of Positive Symptoms (SAPS): The SAPS assesses severity of positive symptoms like hallucinations, delusions, and the total score of the SAPS is 170. It was developed by Andreasen in 1990 and its Turkish validity and reliability study was conducted by Erkoç et al. in 1991 [15, 17].

Morisky 8-item medication adherence questionnaire (MMAS-8): Good medication adherence is a key factor on the treatment response to the antipsychotics in schizophrenia. There is a high rate of low treatment adherence in patients with schizophrenia, and low adherence is one of the most important reasons of poor clinical course in schizophrenia [3]. The MMAS-8 assesses if there is a poor compliance to medication intake because of forgetfulness, carelessness or stopping the drug voluntarily [2, 7]. It is a self-rated questionnaire consisting of eight questions and first seven elements of the questionnaire include yes/no answers while the eighth element has five answers [18]. When the answer indicates a negative adherence issue, a score of 1 is recorded. A score of 3 and higher on the MMAS-8 indicate a poor medication adherence in patients with schizophrenia while a score of 2 and lower on the scale show a good medication adherence. It was developed by Morisky et al. [18] and Turkish validity and reliability study was performed by Hacıhasanoglu Asılar et al. in 2014 [18, 19].

Schedule for Assessing Insight Scale (SAI): The SAI is a likert scale and it is used to measure the insight level of the patients. Its items are rated from 0 to 2 and it has 7 items. It was developed by David et al. [20] and its Turkish validity and reliability study was made by Arslan et al. in 2000 [20, 21].

Calgary Depression Scale for Schizophrenia (CDSS): The CDSS is a nine-item structured interview scale, and it is used to measure the depression level within last two weeks in patients with schizophrenia. It was developed for the assessment of depressive symptoms by Addington et al. [22]. Turkish validity and reliability study were made by Oksay et al. in 2000 [22, 23].

Global Assessment of Functioning Scale (GAF): Functionality in schizophrenia is defined as the ability to work full-time, to have good social relationships and communication skills, and being able to accomplish daily tasks by himself/herself in society. The GAF scale is a widely used scale for assessment of the functionality level of the patients with schizophrenia and it was used in the present study. The scale is scored between 0 to 100, and it was developed by Endicott et al. [24] and it was included in Diagnostic and Statistical Manual of Mental Disorders (4th ed.; DSM-IV) [24, 25].

**Statistical Analysis**

Clinical data were presented as mean ± standard deviation (SD). Demographic and clinical characteristics of the patients with good and poor adherence were compared with descriptive statistics. Pearson correlation analysis was used to

assess clinical correlations of the treatment adherence and insight level. Logistic regression analysis was carried out to determine which clinical variables were more predictive on the treatment adherence. The statistical analyses were carried out with the Statistical Package for the Social Sciences (SPSS) version 21. All probability values (p values) were computed as two - sided, and $p< 0.05$ was considered statistically significant.

## Results

The sample included 229 patients with a mean age of 38.96 ± 10.36 years, and 30 % (n= 69) of the sample was female while 28 % (n=64) of the individuals were married. About 24 % (n=55) of the patients was classified as having poor medication adherence according to the MMAS-8 scale while 76 % (n=174) of the patients showed good medication adherence. The poor adherence rate was 13 % in female patients, whereas it was 29 % in male patients. The difference in adherence rate between the male and female was significant ($p< 0.05$). Mean onset age of the disorder was 23.50±6.41years, and mean duration of the disorder was 15.49±6.41 years in the patients with good adherence. On the other hand, they were 21.91±5.09 and 15.73±8.49 years in the poor adherence group. Comparison of the onset age, duration of the disorder, and CDSS score did not show a significant difference between the groups ($p> 0.05$). In addition, no significant difference was seen between the two groups in terms of age, marital status, and education level ($p> 0.05$). Poor treatment adherence was associated with male gender, more severe BPRS, SANS, SAPS scores, and lower functionality level ($p< 0.05$). The BPRS scores were 26.45 ± 5.86 and 21.97 ± 8.27 in poor and good adherence groups, respectively ($p< 0.05$). Also, the patients with poor adherence demonstrated more severe positive and negative symptoms compared to those with good adherence (SAPS: 32.82 ± 6.85, 28.16 ± 7.85; SANS: 32.51 ± 6.28, 28.50 ± 7.06, respectively, $p< 0.05$). However, mean equivalent dose of chlorpromazine and severity of the depressive symptoms did not show a significant difference between the patients with poor and good adherence ($p> 0.05$). Table 1 shows the comparison between the two groups in terms of the main sociodemographic and clinical characteristics.

The SAI score was higher in good adherence group and the difference between two groups was significant (10.03 ± 1.72, 9.11 ± 1.44, respectively, $p< 0.05$). Age, gender, marital status, and education level were not associated with the insight level ($p> 0.05$). The SAI score was negatively correlated with duration of the disorder and mean antipsychotic dose; but positively correlated with advanced age of onset and higher GAF score. The severity of BPRS, SAPS, and SANS symptoms were all negatively correlated with the SAI score (r =-0.481, r =-0.402, r =-0.413, respectively, $p< 0.01$). The correlation of the insight level with clinical characteristics of the patients is shown in Table 2.

Logistic regression analysis was carried out to determine which clinical variables were more predictive on the treatment adherence. The logistic regression analysis including the SANS, SAPS, SAI, and GAF scales revealed that functionality level (Odds ratio (OR)=4.158, p = 0.041, B =0.065) was more predictive on poor adherence. The logistic regression analysis is demonstrated in Table 3.

## Discussion

Nearly one-fourth of the patients with schizophrenia showed poor medication adherence. Sociodemographic variables did not show a significant difference between the poor and good adherence groups. Severity of clinical symptoms was related with poor treatment adherence and other variables associated with poor adherence were low functionality level and male gender. The patients with good medication adherence had higher insight level

Table 1. Comparison of the sociodemographic and clinical characteristics between the patients with good and poor treatment adherence.

| | Patients with good adherence (n= 174) | Patients with poor adherence (n= 55) | p | |
|---|---|---|---|---|
| Age (year) [a] | 39.30±10.11 | 37.89±11.15 | 0.177 | F=1.832 |
| Gender [b] | | | 0.011* | $x^2$= 6.517 |
| Female | 60 (34.5) | 9 (16.4) | | |
| Male | 114 (65.5) | 46 (83.6) | | |
| Marital Status[b] | | | 0.245 | $x^2$=1.350 |
| Single or Seperated | 122 (70.1) | 43 (78.2) | | |
| Married | 52 (29.9) | 12 (21.8) | | |
| Education level[b] | | | 0.527 | $x^2$= 2.227 |
| Primary school or lower | 53 (30.5) | 12 (21.8) | | |
| Middle school | 45 (23) | 19 (34.5) | | |
| High school | 57 (32.8) | 18 (32.7) | | |
| University | 19 (10.9) | 6 (10.9) | | |
| Onset age[a] | 23.50±6.41 | 21.91±5.09 | 0.094 | F= 1.768 |
| Duration of schizophrenia[a] | 15.49±6.41 | 15.73±8.49 | 0.850 | F=0.084 |
| Number of hospitalizations[a] | 2.75±1.86 | 3.44±2.25 | 0.057 | F=2.271 |
| SANS[a] | 28.50±7.06 | 32.51±6.28 | 0.000* | F=0.743 |
| SAPS[a] | 28.16±7.85 | 32.82±6.85 | 0.000* | F= 2.606 |
| BPRS[a] | 21.97±8.27 | 26.45±5.86 | 0.000* | F=8.908 |
| GAF[a] | 57.24±7.06 | 52.36±5.92 | 0.000* | F=1.459 |
| CDSS[a] | 1.76±1.90 | 1.71±1.23 | 0.906 | F=0.143 |
| SAI[a] | 10.03±1.72 | 9.11±1.44 | 0.000* | F=2.087 |

Data; number of cases (percentage). # Mean±S.D. a Independent sample t-test, b Chi-square test for independence, $p < 0.05$ * Scale for the Assessment of Positive and Negative Symptoms (SAPS/SANS), Brief Psychiatric Rating Scale (BPRS), Calgary Depression Scale for Schizophrenia (CDSS), Schedule for Assessing the three components of insight (SAI), and Global Assessment of Functioning Scale (GAF).

compared to those with poor adherence. Insight level was also not associated with the sociodemographic variables. Although onset age of schizophrenia and duration of the disorder were not related with treatment adherence, lower insight level was associated with earlier age of onset and longer duration of schizophrenia. Severity of positive, negative, and general psychiatric symptoms showed a moderate negative correlation with insight level. Functionality level demonstrated the highest correlation with insight level, and it was found to be more predictive on poor treatment adherence.

Table 2. Correlation of the insight level with clinical characteristics of the patients.

| Variables | p | r |
|---|---|---|
| Age | 0.402 | -0.056 |
| Onset age | 0.017 | 0.157 |
| Duration of schizophrenia | 0.009 | -0.172 |
| Number of hospitalizations | 0.223 | -0.095 |
| SANS | <0.000 | -0.413 |
| SAPS | <0.000 | -0.402 |
| BPRS | <0.000 | -0.481 |
| CDSS | 0.305 | 0.109 |
| GAF | <0.000 | 0.541 |

Pearson Correlation Analysis, $p < 0.05$* SAPS: Scale for the Assessment of Positive Symptoms, SANS: Scale for the Assessment of Negative Symptoms, BPRS: Brief Psychiatric Rating Scale, CDSS: Calgary Depression Scale for Schizophrenia, SAI: Schedule for Assessing the three components of insight, GAF: Global Assessment of Functioning Scale.

The rate of poor treatment adherence was 24 % in patients with schizophrenia, and there is a great need to plan more preventive strategies on poor adherence. Treatment

adherence consists of adherence to medical treatment and compliance to suggestions of the medical team. Our study assessed medication adherence and it is the most researched issue of treatment adherence. As known, maintenance of recovery can be provided with good adherence to medical therapy in all chronic disorders, however, poor adherence is common in chronic disorders [26,27]. Medication adherence is the core of the schizophrenia treatment and poor treatment adherence in nearly one-fourth of the patients demonstrated a substantial problem in the present study [5]. On the other hand, our study did not assess compatibility with suggestions of the healthcare staff, which is more complex part of the adherence issue.

Table 3. Logistic regression analysis for variables associated with poor adherence.

| Variables | Exp (B) | Standard Error | Wald | p value |
|---|---|---|---|---|
| SANS | 0.011 | 0.035 | 0.100 | 0.752 |
| SAPS | 0.035 | 0.030 | 1.423 | 0.233 |
| GAF | -0.065 | 0.032 | 4.158 | 0.041 |
| SAI | -0.146 | 0.017 | 1.566 | 0.211 |

SAPS: Scale for the Assessment of Positive Symptoms, SANS: Scale for the Assessment of Negative Symptoms, BPRS: Brief Psychiatric Rating Scale, CDSS: Calgary Depression Scale for Schizophrenia, SAI: Schedule for Assessing the three components of insight, GAF: Global Assessment of Functioning Scale.

No significant differences were seen in terms of marital status and education level between the patients with good and poor adherence. It was not surprising as there was no significant relationship between treatment adherence and those variables in most of the previous studies [1,6]. There was no significant relationship between age and treatment adherence in the present study, however, some studies found that nonadherence was significantly associated with younger age [28]. Cultural factors and higher family support in our country might have provided a better medication adherence in younger age. In addition, the poor adherence rate was 13 % in female patients, whereas it was 29 % in male patients. The finding of higher rate of poor adherence in male patients was shown in some previous studies [29]. It was suggested that female patients tend to have more enduring and connected social relationships, and thus, they showed better treatment adherence in those studies [29,30]. In addition, seeking help from others was more common in female patients compared to male patients.

In terms of clinical variables, poor treatment adherence was associated with more severe positive, negative, and general psychiatric symptoms. Lower functionality level was related with poor adherence while onset age of schizophrenia and duration of the disorder did not show a significant association with medication adherence. Those findings of more severe clinical symptoms in the patients with poor medication adherence confirmed the results of previous studies [6,11]. As shown in previous studies, poor treatment adherence is a substantial variable on the clinical course of schizophrenia and causes relapses in many patients with schizophrenia [31]. On the other hand, there was no significant difference in severity of the depressive symptoms between the patients with poor and good treatment adherence. This finding indicated that severity of the depressive symptoms was not associated with psychotic symptom severity, and affective symptoms showed a distinct clinical pattern in patients with schizophrenia [10].

Age, gender, marital status, and education level were not associated with the insight level, and that finding was consistent with most of the previous studies [7,8]. There was a significant difference in insight level between the patients with poor and good adherence, and the good adherence group had higher insight level. In addition, the severity of positive, negative, and general psychiatric symptoms was all negatively correlated with the insight score. Therefore, the present study supported the approval that insight level is a predictive factor on treatment adherence, and closely associated with the severity of clinical symptoms [32]. Many previous studies reported that OCD patients with poor insight show poor treatment adherence, more resistant symptoms, and more severe clinical symptoms [33]. Furthermore, duration of OCD was found to be related with lower insight level in several studies, and the insight level was also negatively correlated with the duration of the disorder in the present study [34]. Therefore, it can be suggested that there is a close similarity between OCD and schizophrenia regarding the relationship between lower insight level and clinical course of the patients.

The insight level was negatively correlated with mean duration of the disorder and mean antipsychotic dose. As schizophrenia causes a gradual cognitive impairment during the clinical course, duration of the disorder and longer antipsychotic use might have affected the insight level [5,35]. Besides that, it can be suggested that low insight is also predictive on mean antipsychotic dose in schizophrenia, considering its predictivity on the clinical severity. The insight level showed a positive correlation between advanced onset age and higher functionality level of the patients. That finding was also similar with previous studies [7,8].

The present study has some limitations. It was designed as a cross-sectional study and the clinical associations cannot be presented in a causal relationship. Additionally, effectiveness of the psychotherapeutic interventions on the treatment adherence and insight were not assessed due to the cross-sectional design. Treatment adherence was not assessed in a particular period and was not confirmed with a blood sample test.

It was noteworthy that medication adherence and insight both showed a high predictivity for functionality of the patients. Furthermore, functionality level was found more predictive on treatment adherence compared to severity of positive and negative symptoms. Therefore, psychotherapeutic interventions should be implemented to increase treatment adherence and insight in schizophrenia even if the psychotic symptoms show resistance [31]. Finally, a complete treatment adherence and full insight level in patients with schizophrenia cannot be achieved, nevertheless, improving treatment adherence and insight might provide better clinical and functional outcomes [36]. Future studies are needed to clarify clinical correlates of the treatment adherence and insight in patients with schizophrenia.

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# Photodynamic action of chlorin e6 against *methicillin resistant staphylococcus aureus* with the aid of ethanol

## Klorin e6'nın etanol yardımıyla *metisiline dirençli stafilokok aureus* üzerindeki fotodinamik etkisi

**Nermin Topaloğlu [1], Emel Bakay [1], Aziz Kolkıran [2]**

Abstract

Aim: The random use of antimicrobials for years has led to bacterial DNA mutation and a result of that, bacteria have become resistant to antibiotics. Methicillin-resistant Staphylococcus aureus (MRSA) is among these types of resistant bacteria that can easily infect when the immune system of the host is suppressed, and it significantly delays the wound healing. Different treatment methods are being investigated to overcome this problem. Antimicrobial photodynamic therapy is a candidate to become an alternative treatment for the destruction of MRSA. The aim of this study was to investigate the effect of chlorin e6 for the photoinactivation of MRSA and the synergetic role of ethanol in this mechanism.

Methods: 655 nm laser light and Chlorin e6 as photosensitizer were examined for the photoinactivation of MRSA. Besides, 20% ethanol was used to increase the total antimicrobial efficacy with lower light energy densities and photosensitizer concentrations. The colony counting method was used to determine viable bacterial cells after each application.

Results: 25 J/cm2 energy density with 20 μM Chlorin e6 and 50 J/cm2 energy density with 10 μM Chlorin e6 showed the highest bactericidal activity. When 20% ethanol was used as an adjuvant, 25 J/cm2 energy dose with 2 μM Chlorin e6 resulted in a better killing effect.

Conclusion: Chlorin e6-mediated photodynamic therapy was successful to destroy MRSA and the addition of ethanol provided the opportunity to obtain higher antibacterial activity with lower light intensities and photosensitizer concentrations.

Keywords: Antibacterial photodynamic therapy, chlorin e6, ethanol, staphylococcus aureus

Öz

Amaç: Antibiyotiklerin uzun yıllar boyunca kontrolsüz bir şekilde kullanılması bakteriyel DNA mutasyonuna yol açmıştır ve bunun sonucunda bakteriler antibiyotiklere dirençli hale gelmiştir. Metisiline dirençli Stafilokok aureus (MRSA) bakterileri, bu tür dirençli bakteriler arasında olup vücudun bağışıklık sisteminin düşmesi sonucu kolayca enfeksiyona sebep olabilmekte ve yara iyileşmesini önemli ölçüde geciktirmektedirler. Bu sorunun üstesinden gelmek için farklı tedavi yöntemleri araştırılmaktadır. Antimikrobiyal fotodinamik tedavi enfeksiyonların yok edilmesine yönelik alternatif bir tedavi olmaya adaydır. Bu çalışmanın amacı ise klorin e6'nın MRSA'nın fotoinaktivasyonu üzerindeki etkisini ve bu mekanizmada etanolün sinerjik rolünü araştırmaktır.

Yöntemler: Bu çalışmada MRSA'nın fotoinaktivasyonu için 655 nm lazer ışığı ve fotosensitizan olarak Klorin e6 incelenmiştir. Ayrıca, % 20 etanol kullanımıyla mekanizmanın antimikrobiyal etkinliği düşük ışık enerjisi yoğunlukları ve fotosensitizan konsantrasyonları ile arttırılmaya çalışılmıştır. Her uygulamadan sonra canlı bakteri hücre sayısını belirlemek için koloni sayma yöntemi kullanılmıştır.

Bulgular: Uygulamalar arasında 20 μM Klorin e6 ile 25 J/cm2 enerji yoğunluğu ve 10 μM Klorin e6 ile 50 J/cm2 enerji yoğunluğu en yüksek bakterisidal aktiviteyi sağlamıştır. %20 etanolün mekanizmaya eklenmesiyle en etkili fotosensitizan konsantrasyonu 2 μM'a düşürülerek 25 J/cm2 enerji yoğunluğu ile birlikte daha etkili bir sonuç elde edilebilmiştir.

Sonuç: Klorin e6 aracılı fotodinamik tedavi, MRSA'yı yok etmekte başarılı olmuştur ve etanol ilavesi, daha düşük ışık yoğunluğu ve fotosensitizan konsantrasyonu ile fotodinamik tedavide daha yüksek antibakteriyel aktivite elde etme fırsatı sağlamıştır.

Anahtar Kelimeler: Antibakteriyel Fotodinamik Terapi, Klorin e6, Etanol, Stafilokok aureus

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## Introduction

Bacterial infections can cause serious problems in different types of wounds, prolong the wound healing process, and may spread. Thus, infections must be eliminated for complete wound healing. Wound infections are common and may result in morbidity and mortality. Methicillin-resistant Staphylococcus aureus (MRSA) infections, which are common among these types of infections in the hospital and the environment, are increasing day by day [1].

MRSA is a gram-positive bacterial species, found in skin and mucosa by infecting the human body. MRSA, which is usually found in the hospital environment, has been easily transferred between patient-doctor and patient-patient, thus it has become a common pathogen in the hospital and community [2, 3]. Staphylococci are common bacteria of the skin and mucous membranes. They are usually present in high numbers in these parts of the body compared to other microorganisms [4]. Therefore, it can cause a rapid pathogenic effect in areas where the immune system is weakened such as after surgical operations. More than 90% of the chronic wounds are bacteria-borne diseases that occur in the oral mucosa, the enteric tract, and the superficial areas that damage the healing mechanism [5]. MRSA and similar bacteria can appear and infect in any situation where immune system elements such as B cell, T cell, antibodies, and neutrophils are repressed [6, 7].

MRSA also has a fast DNA repair mechanism against applied antiseptic or antibacterial applications. Therefore, they can easily resist the treatment mechanisms applied. Because of these reasons, researchers have gone on to explore and develop various applications to eliminate the toxic effects created by MRSA. They have carried out various studies on wound infections. Among these studies, the most common and traditional method has been antibiotic treatment [8]. From the past to today, different bacterial agents have been used in the treatment of bacteria. The result of the production of various bacterial toxins has had different effects on the host immune response. In general, agents have been developed to disrupt the synthesis of bacterial cell walls and other important cell organelles such as genetic material [9]. In the medical world, the discovery of antibiotics has made possible the treatment of many microorganism-borne diseases since the 20th century and antibiotics play important role in many fields [10]. Over time, extensive use of antibiotics and rapid mutations of microorganisms against antibiotics has resulted in antibiotic-resistant microorganisms and these rapid mutations have increased the resistance of these microorganisms [11]. Bacterial resistance is a very important issue as antibiotics become ineffective as bacteria develop ways to counter antibiotics. In this way, the lethal pathogenic bacteria develop and regenerate in poor conditions [12]. As a result of the different and widespread use of antibiotics by humans, many bacteria have become resistant to antibiotics used and have had fatal consequences [13, 14]. MRSA is anxiously resistant bacteria nowadays because of its unique virulence, its ability to cause various infections, and its ability to adapt easily to different environmental conditions and have become resistant to many antibiotics with different mechanisms [15, 16]. When the resistance mechanisms that occur in MRSA are examined in general; it may limit the drug intake, modify the drug target, perform horizontal gene transfer, provide enzymatic inactivation of the drug, or provide the active efflux of the drug to render the administered antibiotic ineffective [17]. Despite the recent development of new antibiotics, MRSA will continue to develop rapid resistance to existing antibiotics. Therefore, antibiotic therapy does not seem to be a definite recovery [18]. For this reason, researchers have begun to develop alternative and effective solutions instead of traditional antibiotic treatment. The destruction of microorganisms by antimicrobial photodynamic therapy (aPDT) is an effective and widely prescribed alternative technique today.

aPDT, one of the most innovative and promising approaches used in this study, is a valuable therapeutic approach for the elimination of MRSA infections. It is a specific method involving the interaction of non-toxic drug or dye, which is known as photosensitizer (PS), with light at the appropriate wavelength [10]. After light irradiation, the PS jumps from low energy levels to high energy levels and becomes excited. The PS transmits the energy to the bio-macromolecules around it through molecular interaction. High energy transferred from PS to available oxygen molecules forms various free radicals or reactive oxygen species (ROS). These products cause irreversible damage to the bacterial cell [19]. This lethal effect may be in the form of lipid membrane degradation or deterioration of single or double-stranded DNA [11]. The rate of formation of ROS and mechanism of action of aPDT is thought to depend on the duration of application of the PS, its localization on/in the cell, and the biological environment applied [19]. aPDT needs three main elements; oxygen, light, and PS. Among these elements, PS directly affects the activity of aPDT [20]. To activate the PS at maximum level, it should have the characteristics of high chemical stability, good solubility in water, low dark toxicity, high affinity for microbial cells, preferential accumulation around the pathogenic microorganism, selectively targeting specific cells and high photo-toxicity under light illumination. Porphyrin derivatives, chlorins, phthalocyanine, Rose Bengal, phenothiazines are commonly used as PSs [11]. Mono-L-Aspartyl-Chlorin e6 (Ce6) is a second-generation PS with chemical purity, low dark toxicity, and easy to synthesize with minimal side effects. It also has the maximum absorption under the red light that is found in the visible region of the electromagnetic spectrum [21]. Since the light absorption capacity of chlorin and its derivatives is at maximum after the irradiation with light in the red portion (600-700 nm) of the visible spectrum, it has been reported that their activity to produce ROS in the tissue is high in this window after exposure to red light [22]. For these properties to provide the expected effect on living microorganism, the chemical properties of the solvent that is used to dissolve Ce6 play a big role in photoinactivation process. Solvents that increase the capacity to generate more ROS in the environment can be preferred where Ce6 and bacterial cells meet, interact, and then this interaction results in cell death. Less polar solvents such as ethyl alcohol have been reported to increase the antibacterial properties of Ce6 [23]. It is also known that ethyl alcohol alone causes destruction and death on microorganisms [24]. EtOH has an increased antimicrobial activity in the presence of water and has two main mechanisms of action that create membrane damage and protein denaturation on the bacterial cell. In membrane damage mechanism, the bacterial membrane integrity is impaired due to the dissolution of membrane lipids in the presence of EtOH. In the protein denaturation mechanism, EtOH causes the proteins in the cell to become dysfunctional. Thus it affects cell metabolism and results in cell lysis at the end [25]. Besides, Pronchnow et al. claimed that the presence of EtOH reduces the PS aggregation rate compared to water, resulting in high singlet oxygen production, and that the half-life of the produced singlet oxygen in EtOH is 5 times more than the half-life of singlet oxygen produced in the presence of water [26]. Thus, EtOH seems to be a good adjuvant to increase the efficacy of the photoinactivation mechanism because of its antimicrobial properties and being a proper PS solvent for the high quantum yield of singlet oxygen [25, 26].

In this study, it was aimed to analyze the possible effect of Ce6 as a PS for the photoinactivation of MRSA and the synergistic action of ethanol to improve the mechanism of aPDT with Ce6. Because of the low toxicity, easy synthesis and production, fast and selective accumulation in the target tissue, and high photosensitizing efficacy, it is thought to be effective in obtaining efficient results in aPDT applications on MRSA. To activate Ce6, 655-nm laser light was used as a light source. Then optimum parameters such as energy dose and PS concentration were determined to destroy MRSA efficiently. In the second part of this study, 20% Ethanol (EtOH) was used as an adjuvant to increase the bactericidal effect of aPDT by lowering the levels of energy dose and Ce6 concentration.

## Material and methods

### Bacterial Strain

A clinical isolate of MRSA strain was used to analyze the bactericidal effect of aPDT. MRSA from -80°C frozen stock was used in the streaking method to obtain single colonies. Before each experiment, a single colony of MRSA was incubated in tryptic soy broth (TSB) at 37°C for 18-24 hours. And then the suspension was centrifuged and the supernatant was discarded. Centrifuged bacteria were suspended in phosphate-buffered saline (PBS) and made ready for application to be around 108 CFU/ml.

### Photosensitizer and Ethanol

In this study, the Ce6 agent (Santa Cruz Biotechnology, Dallas, TX, USA), which is in the cationic structure and the chlorin class, was used as PS. Ce6 that has C34H36N4O6 molecular formula and 536.684 g/mole molecular weight is a second-generation drug that can be used in aPDT applications. Ce6 solutions have been prepared and kept in the dark because of the photobleaching problem of the PS in the light environment. It was dissolved in PBS and applied freshly for each experiment. 1, 2, 5, 10, and 20 μM Ce6 concentrations were used throughout this study.

EtOH was used as an adjuvant to increase the effectiveness of Ce6. The Ce6 was dissolved in 20% EtOH which was obtained by mixing absolute EtOH with distilled water. Ce6 solutions in 20% EtOH were prepared at specific concentrations. These solutions were examined at different levels of activity with light applications.

### Optical Setup

A diode-pumped laser device emitting red light at 655 nm of wavelength was used as a light source (PS4 III.LED, Changchun New Industries Optoelectronics Co. Ltd., Changchun, China). The fiber optic which was used to deliver the light to the cells was placed perpendicularly to the 96-well plate where bacteria were seeded on the optical table. The distance between the optical table and the fiber tip was set to 8.7 cm. The illumination area was 3.14 cm2 on the optical table. The output power of the light from the optical fiber is 200 milliwatts (mW) and the power density was 63 mW/cm2. This diode-pumped laser device has a Gaussian beam distribution. To irradiate the cells homogenously with laser light, the core part of the light was used as the illumination area and the energy density of the laser beam was checked by a power meter (Thorlabs, Germany) before each light applications. To obtain the desired antibacterial effect, the optimum laser energy doses were determined by keeping the power density constant and changing the application time. The light intensities applied were 25 and 50 J/cm2.

### Experimental Procedure

Six different main groups were formed in the aPDT study using different drug concentrations and different combinations of light energy doses.

1. “Control Group” No light or PS was applied,
2. “Laser Group” Only the laser was applied,
3. “Ce6 Group” Only PS was applied,
4. “EtOH Group” Only 20% EtOH was applied
5. “aPDT Group” Light and PS were applied together,
6. “aPDT - EtOH Group” Light is applied together with Ce6 dissolved in 20% EtOH,

At the beginning of each experiment, 50 μL bacterial solutions were seeded on 96-well plates. All the applications were performed on these plates. After the addition of bacterial solution, the following steps were performed; (1) An equal volume of PBS was mixed with the bacterial solution in Control and Laser groups. (2) An equal volume of Ce6 solution was mixed with the bacterial solution in Ce6 and aPDT groups. (3) An equal volume of Ce6 solution in 20% EtOH was mixed with the bacterial solution in only EtOH and aPDT-EtOH groups. (4) Then the bacteria were incubated with these solutions for 15 minutes. (5) After incubation, the light was irradiated on bacteria in Laser, aPDT, and aPDT-EtOH groups. (6) When these applications were completed, the serial dilution method was performed to determine the number of live and dead bacterial cells.

### Statistical Analysis

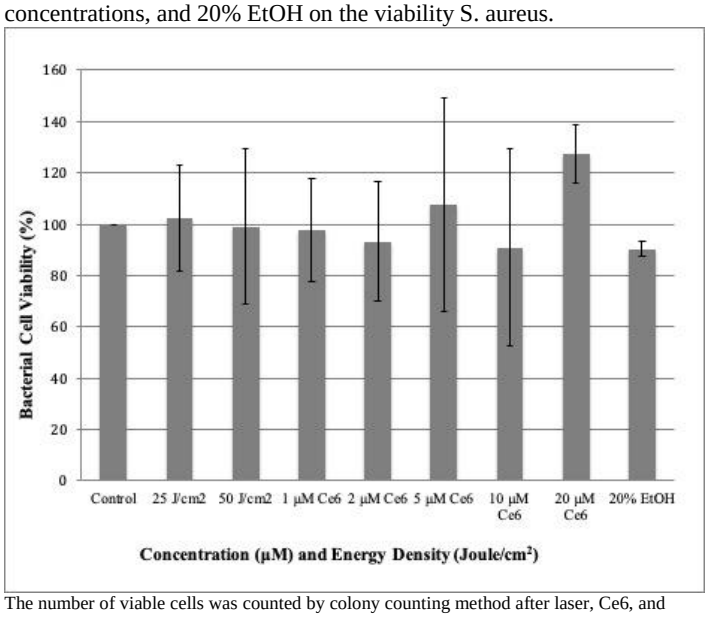
Each experimental group was examined with 3 samples and repeated at least 3 times. All the data obtained from these experimental groups were normalized by the data of the control group. These normalized data were firstly analyzed by one-way ANOVA and then each experimental group was compared with the control group by the Student’s t-test. The statistical difference was determined as $p < 0.05$.

## Results

### The Effect of Chlorin e6, Light and 20% EtOH on MRSA

In this study, aPDT with Ce6 was examined on MRSA and then the role of EtOH was analyzed in Ce6-based aPDT applications. First of all, MRSA was incubated with different Ce6 concentrations (1, 2, 5, 10, and 20 μM) to examine whether Ce6 has any dark toxicity on bacteria or not. In these groups where only the PS was applied, similar results were obtained with the control group. Maximum reduction in cell viability was observed with 10 μM Ce6 and it was approximately 9% which cannot be considered as meaningful dark toxicity. Besides, 20 μM Ce6 concentration caused an increase in the bacterial cell population with a rate of 27%. These results showed that only Ce6 application did not have any lethal effects on MRSA bacterial strain (Figure 1). Then the effect of two different energy doses (25 and 50 J/cm2) was analyzed on MRSA. 25 J/cm2 light intensity caused a slight increase in cell number. On the other hand, 50 J/cm2 resulted in only a 1% decrease. Any of them cannot be considered as an effective treatment on bacterial cells. According to these results, it was understood that only laser application with these energy doses had no lethal effect on MRSA, too (Figure 1). Before aPDT applications, the effect of 20% EtOH was also examined to understand its antibacterial effect on MRSA. In only 20% EtOH-treated groups, cell viability decreased by nearly 10%. When it was compared with the control group, it was understood that the effect of 20% EtOH did not cause any statistically significant difference in cell viability (Figure 1).

Figure 1. Bactericidal activity of different light doses, Ce6 concentrations, and 20% EtOH on the viability S. aureus.

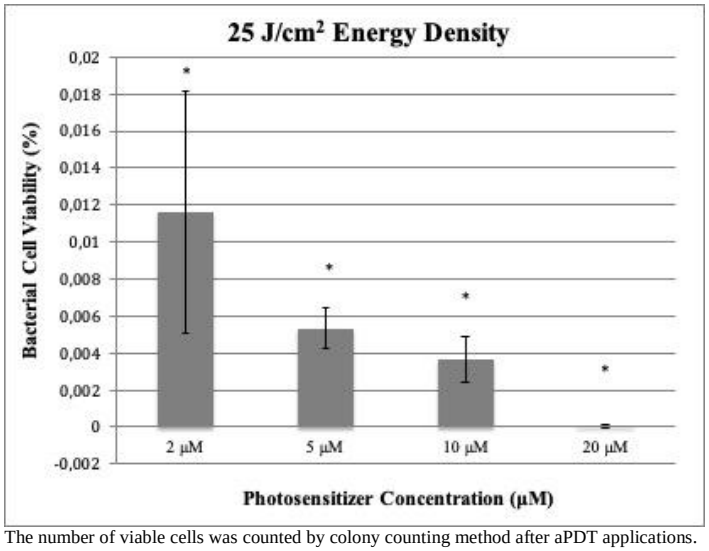



The number of viable cells was counted by colony counting method after laser, Ce6, and EtOH applications. Data of each experimental group were normalized with the data of the control group (Light dose: 25 and 50 J cm2 and Ce6 concentrations: 1, 2, 5, 10, and 20 µM).
* shows the statistical significance with respect to the control group and p-value smaller than 0.05 was considered as statistically significant (n ≥ 8).

**The Photoinactivation with Ce6 on MRSA**

In aPDT applications, 25 J/cm2 light energy was examined together with 4 different Ce6 concentrations (2, 5, 10, 20 µM). Any of these combinations were successful to eradicate MRSA with more than 99% mortality rate and they were statistically significant when they were compared with the data of the untreated control group. The most efficient application with a rate of 99.99% was obtained with 25 J/cm2 energy dose and 20 µM Ce6 concentration (Figure 2).

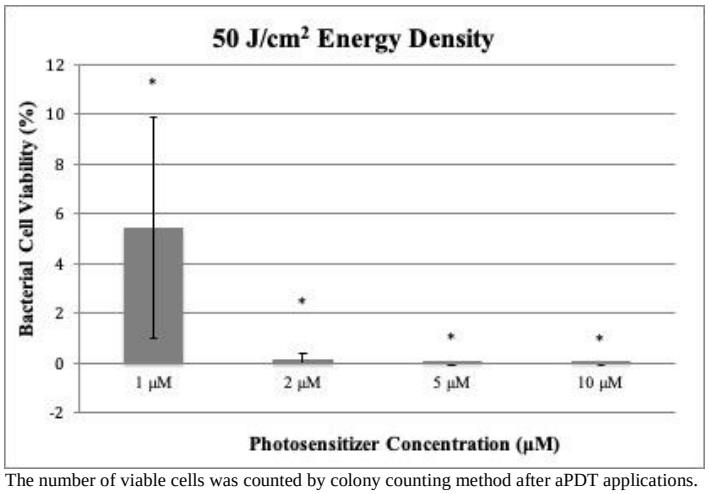
Figure 2. Bactericidal activity of different aPDT doses on the viability of S. aureus.




The number of viable cells was counted by colony counting method after aPDT applications. Data of each experimental group were normalized with the data of the control group (Light dose: 25 J cm2 and Ce6 concentrations: 2, 5, 10, and 20 µM).
* shows the statistical significance with respect to the control group and p-value smaller than 0.05 was considered as statistically significant (n ≥ 8).

Then 50 J/cm2 energy dose was applied with 4 different Ce6 concentrations (1, 2, 5, 10 µM) on MRSA. By using more intense light, Ce6 concentration was reduced to its half which was 10 µM to obtain the same bactericidal effect with a rate of 99.99% (Figure 3).

Figure 3. Bactericidal activity of different aPDT doses on the viability of S. aureus.




The number of viable cells was counted by colony counting method after aPDT applications. Data of each experimental group were normalized with the data of the control group (Light dose: 50 J cm2 and Ce6 concentrations: 1, 2, 5, and 10 µM). Each column indicates normalized data ± standard deviation (n>8).
* shows the statistical significance with respect to the control group and p-value smaller than 0.05 was considered as statistically significant (n ≥ 8).

**The Effect of 20% EtOH in Ce6-based Photoinactivation**

To examine the effect of EtOH in the photoinactivation process, Ce6 solutions were prepared in 20% EtOH. 3 different Ce6 concentrations (1, 2, 5 µM) were examined with 25 J/cm2 energy dose. As shown in Table 1, These Ce6 concentrations were capable to create more than 99% antibacterial activity when they were in 20% EtOH solution after the irradiation by 25 J/cm2 laser light. The most effective application was performed with 2 µM Ce6 in 20% EtOH. Thus, the adjuvant effect of EtOH on MRSA has shown a significant lethal effect with less amount of Ce6 under light illumination (Table 1).

Table 1. Percentage decrease in the viability of S. aureus after Ce6-mediated photoinactivation process with/without EtOH.

| Ce6 Concentration (µM) | % Decrease in Cell Viability | | |
|---|---|---|---|
| | aPDT with 25 J/cm² | aPDT with 50 J/cm² | aPDT with 25 J/cm² + 20% EtOH |
| 1 µM Ce6 | - | 94.5381 | 99.9962 |
| 2 µM Ce6 | 99.9884 | 99.8318 | 99.9999 |
| 5 µM Ce6 | 99.9947 | 99.9904 | 99.9988 |
| 10 µM Ce6 | 99.9963 | 99.9990 | - |
| 20 µM Ce6 | 99.9999 | - | - |

## Discussion

In this study, the photoinactivation capability of Ce6 was examined with a 655 nm laser light on MRSA. Then EtOH was used to increase the bactericidal effect of this mechanism by lowering the Ce6 concentration. When any of these parameters which are PS, light, or EtOH was used alone, it desired not to cause any bactericidal activity to limit the side effects of this mechanism. aPDT can be considered as successful when it is applied to the infected area of the biological tissue without giving any harm to the neighboring tissue. Different Ce6 concentrations (1, 2, 5, 10, and 20 µM) did not cause any dark toxicity on MRSA when applied alone. The maximum reduction in cell viability was obtained with 10 µM Ce6 and it was around 9%. When their impacts were analyzed statistically, none of them showed any difference from the untreated control group. Similar results were obtained with the application of only light (25 and 50 J/cm2) and only 20% EtOH treatments. Light treatments resulted in a slight change in bacterial cell viability. 20% EtOH application decreased the cell viability with a rate of

nearly 10%. None of them were statistically different from the control groups. It can be concluded that these parameters cannot cause any significant cell death when they were applied alone.

When the aPDT groups that were received 25 J/cm2 laser irradiation were examined, more than 99% cell death was achieved with any of the Ce6 concentrations. The most successful one was the treatment with 20 μM Ce6 irradiated by 25 J/cm2. This treatment resulted in more than 99.99% cell death. The general purpose of aPDT is to obtain the maximum cell death with minimum light energy dose and Ce6 concentration. Among these parameters, the PS is the most possible toxic element of these applications. So it is always desired to minimize the concentration level of PSs. To increase the bactericidal capacity of PS in aPDT with lower concentrations, light energy dose must be increased [27]. Therefore, light energy was increased to 50 J/cm2 and its effect was examined with 4 different Ce6 concentrations (1, 2, 5, and 10 μM). 1 μM Ce6 concentration caused a cell death with a rate of 94% which was quite high but not efficient to eradicate bacterial population with an acceptable range. When the concentration of Ce6 was increased slightly, aPDT applications resulted in more than 99% cell death. Among the Ce6 concentrations used with 50 J/cm2, the best result was obtained with 10 μM Ce6 concentration.

In aPDT groups using 25 and 50 J/cm2 laser energy doses, the desired more than 99.99% bacterial viability reduction was seen in experimental groups containing 20 μM and 10 μM Ce6 concentrations, respectively. Although 50 J/cm2 is higher energy level when compared to 25 J/cm2, it showed less bactericidal activity when its effect was compared with the effect of PS alone, which means that these energy doses of red light were not as harmful as PS itself. The aim of the work is to achieve maximum bacterial cell death at the minimum laser energy dose and PS concentration and also to avoid the lethal effect of the PS or laser alone. Therefore, the aPDT group containing 50 J/cm2 and 10 μM Ce6 concentration was accepted as the desired doses for the photoinactivation process.

In the second part of this study, EtOH was used as an adjuvant to increase the effect of aPDT with a lesser amount of light dose and PS concentration. In this part, 25 J/cm2 energy dose was examined with 1, 2, and 5 μM Ce6 concentrations. With the addition of 20% EtOH to the mechanism, more than 99.99% cell death was achieved with any of these Ce6 concentrations. The most efficient antibacterial activity was obtained with 2 μM Ce6. The use of 20% EtOH provides the opportunity to decrease PS concentration level in a significant amount (10X reduction according to the application with 25 J/cm2, 5X reduction according to the application with 25 J/cm2). So it will be a promising strategy in aPDT applications to avoid the lethal effect of the drug alone. It is thought that EtOH increases bacterial wall permeability and penetration capability of Ce6 into the cells [26].

In conclusion, the aim of this study was to develop an alternative antibacterial mechanism that can completely destroy MRSA strains. The desired effects were achieved in both EtOH-free and EtOH-containing applications with Ce6 and 655-nm laser light. Significantly high bactericidal effects were obtained at a lower level of red light and Ce6 concentration in this study. Achieving the maximum levels of cell death with such low quantities of Ce6 levels reflects the success of the Ce6-based aPDT with and without EtOH. Thus, using Ce6 as a PS in the presence of 655-nm laser light can be a good candidate for the elimination of local infections caused by MRSA clinically.

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# Perinatal outcome of patients with placenta percreata

## Plasenta perkreatalı hastaların perinatal sonuçları

**Erkan ELCI [1], Sena SAYAN [2], Gulhan ELCI [3], Numan CIM [4]**

Abstract

Aim: Placental invasion anomalies are rare, but it causes serious fetomaternal morbidity and mortality. In our study, we aimed to evaluate the fetomaternal results of cases with Abnormal Invasive Placenta ( Placenta Percreata).

Methods: Patients who underwent a peripartum hysterectomy or whose placenta was left in uterin cavity due to placenta percreata in our hospital were retrospectively analyzed. Data of 20 patients were noted.

Results: The mean age of the patients was ± SD (min-max) 33 ± 5,704 (25-46) and 60% (n = 12) of the patients had additional diseases. Fifty five percent of patients (n=11) were operated in emergency conditions and 45% (n = 9) in elective conditions. Surgical complications were 65% (n = 13) bladder injuries, 30% (n = 6) disseminated intravascular coagulation (DIC), 20% (n = 4) infection, 15% (n = 3) relapartomy and 5% (n = 1) was pulmonary embolism. Mortality increased three times (OR; 3.003 (95% CI, 0.372-24.390) in patients with a comorbidity, while 4.7 times (OR; 4.784) in emergency operations. Operations under elective conditions and previously ultrasonographic diagnosis (Odd ratio values ; 0,219 (95% CI, 0,021-2,447) and 0,615 (95% CI, 0,043-8,695), recpectively) decreased maternal mortality.

Conclusion: Prenatal diagnosis and performing elective surgeries in percreata cases are important to reduce maternal mortality. Although there are various surgical complications, we think that DIC development is important in mortality and massive transfusion does not decrease the mortality.

Keywords: Recurrent caesarean, hysterectomy, placenta percreata

Öz

Amaç: Plasenta invazyon anomalileri nadir görülmesine rağmen ciddi fetometarnal morbidite ve mortaliteye sebep olmaktadır. Çalışmamızda plasenta invazyon anomalisi olan olguların perinatal sonuçlarını değerlendirmeyi amaçladık.

Yöntemler: Hastanemizde 2012-2014 tarihleri arasında peripartum histerektomi yapılan ve intraoperatif plasentası uterin kavitede bırakılan hastalar retrospektif olarak incelendi. 20 hastanın verileri not edildi.

Bulgular: Hastaların yaş ortalamaları ± SD (min-max) 33 ± 5,704. (25-46) saptandı. Hastaların % 60'ında (n=12) ek hastalıklar da vardı. Hastaların % 55'i (n=11) acil şartlarda ve % 45'i (n=9) elektif şartlarda opere edildi. Perkreta operasyonlarında oluşan cerrahi komplikasyonlar sıklığına göre sırasıyla % 65(n=13) mesane yaralanması, % 30 (n=6) dissemine intravaskülar kuagulasion (DIC), % 20 (n=4) enfeksiyöz komplikasyonlar ve % 5 (n=1) pulmoner emboli şeklindeydi. Maternal mortalite riskinin ek hastalığı olan hastalarda 3 kat (OR; 3,003 (95%CI, 0,372-24,390) ve acil şartlarda ameliyat olan hastalarda 4,7 kat (OR;4,784 (95%CI, 0,408-47,619) artığı saptandı. Hastaların elektif şartlarda ameliyat olması ve önceden ultrasonografik olarak tanı almış olmalarının da (OR; 0,219 (95%CI, 0,021-2,447), OR; 0,615 (95%CI, 0,043-8,695) maternal mortalite riskini azalttığı saptandı. Korelasyon analizinde maternal mortalite ile maternal yaş (0,473, p=0,035) ve masive kan transfüzyonunun (0,562, p=0,010) pozitif korelasyon, postoperatif hemoglobin değeri ile de negatif kolerasyon gösterdiği saptanmıştır (-0,723, p= 0,010).

Sonuç: Plasenta perkreatalı hastalarda, prenatal tanı konulmuş olması ve hastaların elektif şartlarda opere edilmesi maternal mortalite riskinin azaltılması açısından önem arz etmektedir. Çeşitli cerrahi komplikasyonlar olmasına rağmen DIC tablosu mortalite açısından önemlidir ve bu tabloda masif transfüzyon da mortaliteyi azaltmamaktadır.

Anahtar Kelimeler: Tekrarlayan sezaryen, histerektomi, plasenta perkreata

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## Introduction

Abnormal Invasive Placenta (AIP) is a life-threatening condition. It is associated with maternal and fetal morbidity and mortality [1]. According to the degree of this abnormal trophoblastic infestation, there are three variants: acreta (after the decidual layer), increta (invasion into myometrium) and percreta (myometrium, uterine serosa, and adjacent tissue invasion) [2]. In general, these invasion anomalies are called placenta acreta. In the current review, placenta acreta ranges between 1 in 100 and 1 in 10,000 [3]. The incidence of placental invasion anomaly has increased 10 times in the last 50 years with the frequency of cesarean (C / S) [4]. In some middle-income countries such as Egypt, Turkey, Mexico, Brazil more than half of the births were achieved by elective C/S [5]. This suggests that in these countries AIP-related mortality and morbidity will increase in the future.

We aimed to retrospectively analyze fetomaternal mortality and morbidity in placenta acreta cases encountered due to the high birth rates in our clinic.

## Material and methods

In this study, 20 patients who underwent peripartum hysterectomy diagnosed with placenta percreata or left in the uterine cavity between 2010 and 2014 in our hospital were retrospectively analyzed. This study conforms with the principles of the 2008 Declaration of Helsinki and written informed consent was obtained from the patient or the patient's legal guardians(Ethics committee approval:(07)-28/05/14).

The demographic characteristics of the patients, age, gravida and parity, gestational week, previous C/S history, previous uterine surgery, the condition of the placenta in the current pregnancy, complaints during admission to the hospital, hospitalization time, intensive care hospitalization time, newborn weight and APGAR score were noted. Abdominal and uterine incision type, hypogastric artery ligation (HAL), complications during the operation, postoperative complications, erythrocyte transfusion, and fresh frozen plasma (FFP), maternal and fetal mortality and morbidity were noted.

Post-operative pathology results were examined. Transabdominal and transvaginal ultrasonography and doppler ultrasonography were used to evaluate the placenta position and degree of invasion. For elective cases, 6 units (U) erythrocyte suspension (ES) and 6 units (U) FFP were prepared before the operation. In elective cases, a midline abdominal incision and a fundal median incision were frequently used. Afterwards, the umbilical cord was clamped. The placenta was left in place and the bladder dissection started. A total hysterectomy (TAH) was performed in those with previa totalis and an inferiorly located placenta. A subtotal hysterectomy (SAH) was performed in cases with a low invasion surface and a placental location far from the cervix. HAL was only performed in patients with prolonged bleeding time, intraoperative excess bleeding, or with a high possibility of reoperation, intraoperative transfusion, and patients who were operated for the second time. In cases where bladder dissection was not performed completely, the bladder was perforated by the surgeon, and dissection was continued. If required, a urology consultation was requested and a double J catheter was inserted into the ureters from the bladder. A drain was placed in all cases. The patients were taken to the adult intensive care unit, if necessary, according to the assessment of the anesthesiologist.

A conservative approach was made to a single case with a previous C/S history and ultrasonographically diagnosed in the prenatal period. The placental cord was clamped and HAL was performed due to abundant organ invasion in the abdomen. The bladder could not be reached and the surgery was discontinued. Metotrexate was then planned to be applied.

**Statistical Analysis**

In this study, statistical analysis was performed with SPSS 22 software (IBM Corp., Armonk, NY, USA). Descriptive statistics of data were expressed with average ± standard deviation, median and other statistics using the explore option in SPSS. maternal mortality odds ratio rates were estimated. Spearman's correlation was used to determine how the mortality is affected by other values. Uncertainty was expressed as a 95% confidence interval (CI). $P<0.05$ was considered statistically significant.

## Results

In our hospital, 5263 birth deliveries were recorded. 1217 (%23,1) of these patients were delivered by C/S. 623 (%51,1) of the C/S deliveries had a C/S history. Twenty of these patients were diagnosed with placenta percreata intraoperatively or after their hysterectomy. Cases without a pathology sample and placenta in the uterine cavity were not considered as placenta percreata.

Table 1. Demographic characteristics of patients, risk factors, maternal and fetal outcomes

| | | | |
|---|---|---|---|
| Age(year) | | Median ± SD (min-max) | 33 ± 5,704 (25- 46) |
| Gravity | | Median ± SD (min-max) | 3 ± 1,182 (2 -7) |
| Parity | | Median ± SD (min-max) | 2 ± 1,218 (1- 6) |
| Symptom | | | |
| | Bleeding | (%, n) | 50%, 10 |
| | Pain | (%, n) | 30%, 6 |
| | Control | (%, n) | 15%, 3 |
| | IUGR | (%, n) | 5%, 1 |
| Medical history | | | |
| none(%,n) | | | 60%, 12 |
| yes(%,n) | | | 40%, 8 |
| | HT | (%, n) | 35%, 7 |
| | GDM | (%, n) | 5%, 1 |
| Smoking | Yes | (%, n) | 65%, 13 |
| | No | (%, n) | 35%, 7 |
| Previous birth | C/S | (%, n) | 95%, 19 |
| | SVB | (%, n) | 5%, 1 |
| Number of caesarean section history | | Median ± SD (min-max) | 2 ± 0,894 (0 -3) |
| Maternal Intensive Care Need | | (%, n) | 55%, 11 |
| Duration of hospitalization (day) | | Mean ± SD (min-max) | 13,35 ± 8,418 (4 -30) |
| Ultrasonographically diagnosed | | (%, n) | 85%, 17 |
| Concomittant plasenta previa. | | (%, n) | 60%, 12 |
| Gestational birth week | | Mean ± SD (min-max) | 36 ± 2,434 (30 -39) |
| Gestational weight (gr) | | Mean ± SD (min-max) | 2611 ± 591,490 (1560 -3600) |
| 1.minute APGAR | | Mean ± SD (min-max) | 5,75 ± 1,482 (3 -7) |
| 5.minute APGAR | | Mean ± SD (min-max) | 9,00 ± 1,338 (7,07 - 13,30) |
| Mortality | | | |
| | Maternal | (%, n) | 25%, 5 |
| | Fetal | (%, n) | ----- |

IUGR: intrauterine growth retardation, HT: hypertension, GDM: gestational diabetes mellitus, C/S: cesarean section, SVB:spontaneous vaginal birth

Table 1 summarizes the demographic characteristics, risk factors, maternal, and fetal outcomes of the patients. Mean age, gravida, parity values of the patients were ± SD (min-max) 33 ± 5,704 (25-46), 3 ± 1,182 (2-7), 2 ± 1,218 (1-6), respectively. The complaints of the patients for rehospitalisation and readmission were noted as 50% bleeding, 30% pain, 15% control

and 5% Intra Uterin Growth Retardation (IUGR). Sixty percent of patients had an additional disease. Hypertension (35%) was the most common in these patients as an additional disease. Except for one patient, previous deliveries were carried out by cesarean method (95%, n = 19). After the operation, 55% of the patients needed intensive care. The average duration of hospitalization of operated patients was 13.35 ± 8.418 days, and 25% of the patients died during their follow-up. A prenatal diagnosis could be made in 85% of the patients by ultrasonography. Although 15% of patients had AIP, the diagnosis could not be made (Table 1). Placenta previa totalis was accompanied by 60% of the patients. The mean ultrasonographic gestational week was determined mean ± SD (min-max) 36 ± 2,434 (30-39). In terms of fetal results; a birth weight mean ± SD (min-max) 2611 ± 591,490 (1560-3600), 1st minute APGAR Mean ± SD (min-max) 5.75 ± 1.482 (3-7), 5th minute APGAR mean ± SD ( min-max) 9.00 ± 1.338 (7.07-13.30) was detected and fetal mortality was not observed.

Table 2. Pre-operative, intra-operative and post-operative patient data

| | |
|---|---|
| Operation | |
| emergency (%,n) | 55%, 11 |
| elective (%,n) | 45%, 9 |
| Skin incision | |
| pfannenstiel(%,n) | 30%, 6 |
| infraumblical(%,n) | 55%, 11 |
| supraumblical and infraumblical(%,n) | 15%, 3 |
| Uterine Incision | |
| fundal vertical (%,n) | 80%, 16 |
| kerr incision(%,n) | 20%, 4 |
| Treatment | |
| TAH(%,n) | 85%, 17 |
| Subtotal TAH(%,n) | 10%, 2 |
| MTX(%,n) | 5%, 1 |
| HAL (%,n) | |
| yes | 60%, 12 |
| no | 40%, 8 |
| Surgical complications | |
| Bladder injury (%,n) | 65%, 13 |
| Infection (%,n) | 20%, 4 |
| DIC (%,n) | 30%, 6 |
| Relaparotomy (%,n) | 15%, 3 |
| pulmonary embolism (%,n) | 5%, 1 |
| Adjacent organ invasion | 60%, 12 |
| Operation duration ( minute) | |
| mean ± SD | 192,50± 52,468 |
| (min-max) | ( 110-240 ) |
| Intraoperative consultation(%,n) | 55%, 11 |
| Blood transfusion (number of patients) (%,n) | |
| yes | 85%, 17 |
| no | 15%, 3 |
| Erytrocyte Suspension Transfusion | |
| Median ± SD | 4,5±3,44 |
| (min-max) | (0-13) |
| FFP transfusion | |
| Median ± SD | 4,5±4,48 |
| (min-max) | (0-16) |
| Pre-op HGB (gr/dl) | |
| Mean ± SD | 10,70± 1,490 |
| (min-max) | ( 7,07-13,30 ) |
| Intra-op HGB (gr/dl) | |
| Mean ± SD | 8,50± 1,173 |
| (min-max) | ( 6,50-10,50 ) |
| Post-op HGB (gr/dl) | |
| Mean ± SD | 8,70± 1,503 |
| (min-max) | ( 5,7-10,0 ) |

MTX: Methotrexate, TAH: Total abdominal Hysterectomy, HAL: Hypogastric artery ligation, FFP: Fresh frozen plasma, ICU: Intensive care unit, USG: Ultrasonography, DIC: Disseminated Intravascular Coagulalopathy, HGB: hemoglobin, Pre-op:Before oparation, Post-op:After oparation

Pre-operative (preop), intra-operative (inop) and post-operative (postop) patient data are summarized in Table 2. 55% of the patients were operated under emergency conditions and 45% were operated under elective conditions. Abdominal incision was infraumblical (IU) in 55% of patients, pfannenstiel (PNS) in 30% and supraumblical (SU) + infraumblical (IU) in 15% chosen. During the operation, 85% Total abdominal Hysterectomy (TAH), 10% SAH were performed. Methotrexate (MTX) treatment was deemed suitable as a conservative method because one patient had advanced adjacent tissue invasion. Adjacent organ invasion was observed in 60% of the patients. HAL was performed in 60% of patients with high bleeding probability and low intraoperative haemoglobin (HGB) Value. Surgical complications were 65% bladder injury, 30% disseminated intravascular coagulation (DIC), 20% infection, 15% relaparatomy and 5% was pulmonary embolism. The operation time was mean ± SD (min-max) 192.50 ± 52.468 (110-240) minutes. Intraoperative consultation (urology, cardiovascular surgery and general surgery) was requested in 55% cases during the operation. Blood and blood product transfusions were performed in 85% of the patients. Transfused ES amount was mean ± SD (min-max) 4.5 ± 3.44 (0-13) and FFP amount was mean ± SD (min-max) 4.5 ± 4.48 (0 -16).

Table 3. Odds ratio rates between maternal mortality and other variables

| | Mortality | Odd ratio (OR) (95%CI) |
|---|---|---|
| HAL (hypogastric arter ligation) | + | 3,496 (95%CI, 0,312 - 38,461) |
| Smoking | + | 2,666 (95%CI, 0,236 - 30,303) |
| Adjacent tissue invasion | + | 3,496 (95%CI, 0,312 - 38,461) |
| Additional disease | + | 3,003 (95%CI, 0,372 - 24,390) |
| Emergency operation | + | 4,784 (95%CI, 0,408 - 47,619) |
| Elective operation | + | 0,219 (95%CI, 0,021 - 2,447) |
| Ultrasonographic preliminary diagnosis | + | 0,615 (95%CI, 0,043 - 8,695) |

Table 3 shows maternal mortality odds ratio values between maternal mortality and other variables. Mortality increased, 3.4 times with adjacent organ invasion (OR; 3.496 (95% CI, 0.312-38.461), 2.6 times if the patient was smoking (OR; 2.666 (95% CI, 0.236-30.303) and 3 times if the patient had a additional disease (OR ; 3,003 (95% CI, 0,372-24,390), 4,7 times (OR; 4,784 (95% CI, 0,408-47,619) in emergency surgery and 3.4 times (OR; 3,496 (95% CI, 0,312) in patients undergoing HAL -38,461)) increases. Although HAL seems to increase mortality, patients undergoing HAL are those with increased bleeding, prolonged surgery, and operated for the second time. The elective operations (OR: 0,219 (95% CI, 0,021-2,447)) and prior ultrasonographic diagnosis (OR: 0,615 (95% CI, 0,043-8,695) reduce maternal mortality. Table 4 summarizes the correlation between maternal mortality and (HGB) values, hospitalization, maternal age, and gestational week. There was a statistically significant correlation between maternal mortality and other variables between maternal age (p = 0.035), massive blood transfusion (p = 0.010), and negative correlation between post-op (HGB) value (p= 0.010). Demographic and operational characteristics of five cases with mortality and data of complications are summarized in Table 5.

Table 4. Correlation between maternal mortality and hemoglobine values, hospitaliation, maternal age and gestational week.

| | Mortality | p |
|---|---|---|
| Age (years) | 0,473* | 0,035* |
| Gravida | 0,053 | 0,804 |
| Gestational week | 0,365 | 0,113 |
| Preoperative hgb (gr/dl) | -0,010 | 0,967 |
| Intraoperative hgb (gr/dl) | -0,060 | 0,800 |
| Postoperative hgb (gr/dl) | -0,723* | 0,010* |
| Operation time | 0,243 | 0,301 |
| Length of hospital stay | 0,443 | 0,051 |
| Transfusion of ES | 0,562* | 0,010* |

* Statistical significance was defined as p< 0.05, ES:Erythrocyte Transfusion

## Discussion

It is reported that the rate of maternal mortality due to AIP can vary between 3% and 10% [6]. In the region where our study was conducted, maternal deaths were reported as 20.1 per 100,000 births and 43 deaths in the year of our study. Five of these deaths are as high as AIP related death and 11% of maternal deaths [7]. Placenta previa, advanced maternal age, multiparity and some uterine interventions have been associated with the incidence of placenta percreata [8]. In addition, the incidence of placental location abnormalities has been shown to increase from 11% to 67% in relation to the number of previous C/S [9]. In 95% of our cases, delivery was previously performed by C/S, only one patient was diagnosed with percreata after NVD, but the patient had a history of curettage. According to average age (33 ± 5,704 (25-46)), there is a statistically significant positive correlation between maternal mortality and age (0.447, p = 0.035). Sixty percent of our cases were accompanied by placenta previa

In the literature, TAH is frequently preferred in treatment, since placenta percreta is associated with placenta previa in most cases and the placenta occupies the lower segment of the uterus [10]. However, it has been reported that performing SAH has many advantages such as fewer injuries to the adjacent organs, less blood loss, and shorter operation time and hospitalization [10]. In our clinic, primarily the SAH or TAH approach is preferred. However, the approach may vary according to the fertility request or surgical difficulty. TAH was performed in 85% of our cases while SAH was performed in 10%. Conservative treatment was planned in only one case due to advanced tissue invasion.

On the other hand, there are also conservative approaches. Intrauterine balloon inflation, methotrexate therapy, uterine, and internal iliac artery ligation, and selective arterial embolization have been described without performing a hysterectomy [11,12]. Furthermore, positive results have been reported in patients with segmental resection of the uterus anterior wall with placenta percreta in selected cases. However, it has been reported that this is not possible for every case that can be determined intraoperatively [13]. In our study, only one case was approached conservatively and an infection developed during the follow-up. In this case, the placenta was completely left inside the uterus, and HAL was performed and Methotrexate was applied. Although the patient received long-term antibiotic therapy, the infection developed. A cure was achieved after 9 months.

It has been reported that HAL has no effect on reducing morbidity, estimated blood loss, and blood transfusion needs [14,15]. The mortality rate of patients with adjacent organ invasion with placenta percreta has been reported to be 7% [16]. We are not doing HAL in all of our cases. However, in cases of prolonged bleeding, prolonged cases, and patients who had been operated for the 2nd time, the HAL operation was performed. This may be the reason for increased maternal mortality in patients with HAL (OR; 3,496).

Complications include bleeding and extensive intravascular coagulation (DIC) development, kidney failure, acute respiratory distress syndrome, infection and bladder, ureter, and / or intestinal injuries [17]. It is emphasized that placing catheters to the ureters reduces the risk of ureteral injury before surgery, and this procedure does not prevent complete injury [18]. Surgical complications seen in our cases were 65% bladder injury, 30% disseminated intravascular coagulation (DIC), 20% infection, 15% relaparatomy and 5% was pulmonary embolism. The reason for all patients with maternal death was DIC and multiple organ failure. We know the primary cause of this was bleeding. A statistically significant negative -0.772 (p = 0.01) correlation was observed between maternal mortality and post-operative HGB values. A statistically significant positive correlation was observed between ES transfusion and maternal mortality(0,562 (p=0,01)). We think that a massive transfusion does not decrease mortality. In deepening cases of invasion, the bladder was perforated iatrogenically and assisted in the anatomical separation of tissues with a ureter catheter inserted from the bladder. The posterior bladder wall bleeds a lot during the operation due to the excessive blood supply at the depth of the invasion [19]. In hemorrhages in this area, when the area is large and the bleeding control becomes difficult, posterior bladderwall resection is performed without wasting time. Surgical procedures in this area appear as fistulas as a long-term complication [20]. While AIP cases in another region with a high birth rate in our country are routinely taken to the intensive care unit after the operation [20], only the patients (55% (n = 11)) that anesthesiologists deem necessary are taken into intensive care in our hospital.

When the newborn findings were examined, the average gestational age was found to be 36 ± 2,434 (30-39) weeks. Accordingly, many studies suggest that preterm C/S should be performed between 340/7 and 356/7 weeks in order to eliminate the risk of bleeding during surgery and thus reduce the rate of maternal morbidity and mortality [19,21]. The advanced

Table 5. Demographic characteristics, complications and surgery data of 5 cases with mortality

| Age (years) | Existence of risk factors | POG (weeks) | Preop Hb (gr/dl) | Sonographically diagnosed | Elective/ emergency | Surgery | USG | Postop complications | Blood Transfusion | ICU stay (days) |
|---|---|---|---|---|---|---|---|---|---|---|
| 41 | yes | 38 | 11,7 | yes | emergency | TAH, HAL, | yes | dic | 10ES 15FFP | 30 |
| 46 | yes | 36 | 7,07 | yes | emergency | TAH, HAL, | yes | dic | 10ES 9FFP | 8 |
| 41 | yes | 39 | 10,2 | no | emergency | TAH, HAL, | no | dic | 13ES 16FFP | 25 |
| 30 | none | 36 | 11,5 | yes | emergency | TAH, HAL, 2$^{th}$OPR. | yes | Dic +sepsis | 8ES 9FFP | 12 |
| 34 | yes | 36 | 11,9 | yes | elective | TAH, HAL, 2$^{th}$OPR. | yes | Dic+sepsis | 6ES 6FFP | 17 |

POG;Period of gestation, TAH;Total abdominal Hysterectomy, HAL;Hypogastric artery ligation, ES:Erythrocyte suspension, FFP:Fresh frozen plasma, ICU;Intensive care unit, USG;Ultrasonography, DIC;Disseminated Intravascular Coagulalopathy, OPR;Operation

gestational week of our cases was attributed to the waiting of the delivery time of patients who could not be diagnosed with percreata. In our study, fetal mortality was not observed, it was found that birth weights were normal compared to the gestational week, but the APGAR score at the first and fifth minutes were lower than normal and were compatible with other studies [22].

Ultrasound is usually the primary tool for evaluating women at risk of AIP, such as patients with placenta previa and previous C/S, while MRI is performed only to confirm the diagnosis or in the event of an inconclusive ultrasound evaluation [23]. The diagnostic values of the two imaging methods were determined to be the same [24]. In previous studies, it was reported that early diagnosis in ultrasonography decreased maternal mortality and blood product transfusion needed [25]. In cases diagnosed before the operation, 85% (n = 17) maternal mortality was observed to decrease (OR: 0.615). In our study, an emergency operation was seen as another important reason for increasing mortality (OR; 4,784) [26]. In our cases, adjacent organ invasion was observed as 60% (n=12) and maternal mortality increased by 3.4 times (OR; 3.496) if adjacent organ invasion was observed. Therefore, diagnosis and taking precautions with prenatal imaging may decrease maternal mortality.

The fact that the number of cases were low and the conservative approach was rather limited due to the previous maternal losses were found to be limitations of our study.

As a result, the incidence of placenta acreta increases with the C/S rate. It is important for a prenatal diagnosis of percreta cases and operation of cases under elective conditions in terms of decreasing maternal mortality. The postoperative hemoglobin value may be important in terms of both hemorrhage estimation and mortality and prognosis. Although there are various surgical complications, we think that DIC development is effective in mortality and that adequate and timely blood transfusion is important instead of a massive transfusion.

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# Neutrophil-to-lymphocyte ratio may guide the choice of treatment in metastatic cancer patients: Chemotherapy or best supportive care

## Metastatik kanser hastalarında tedavi seciminde nötrofil-lenfosit oranı yol gösterici olabilir: Kemoterapi veya en iyi destekleyici bakım

**Serdar Arıcı [1], Ruhper Çekin [1]**

Abstract

Aim: We set out in this study to investigate whether the neutrophil-to-lymphocyte ratio is a predictor in deciding whether to either continue palliative chemotherapy or choose the best supportive care for advanced cancer patients.
Methods: Those with advanced solid tumors who had died after palliative chemotherapy were included the study. The patients were divided into two groups based on the time between the beginning of their last chemotherapy regimen and death, at ≤ 60 or > 60 days. Neutrophil-to-lymphocyte ratio was calculated using the laboratory values taken before the beginning of the last chemotherapy line. The determinant factors of ≤ 60-days survival were examined by logistic regression analysis, and a statistical significance level of alpha was accepted as $p < 0.05$.
Results: The study included 404 patients, with the mean age at diagnosis of 61.7±12.0 years. The mean neutrophil-to-lymphocyte ratio was calculated as 11.3±27.1. In the univariate analysis for determining ≤ 60-days survival, breast and colorectal cancers, ECOG status, single agent chemotherapy usage, neutrophil count, lymphocyte count and neutrophil-to-lymphocyte ratio were all found to be significant factors. The cutoff value determining the ≤ 60-days DCD, was determined as NLR ≥ 3.59. In logistic regression analysis, NLR ≥ 3.59, as well as ECOG status, were found to be significant factors.
Conclusion: The neutrophil-lymphocyte ratio, combined with ECOG, can predict survival in patients with solid advanced tumors and can therefore help clinicians in choosing to either administer chemotherapy to their patients or direct them to the best supportive care.

Keywords: neutrophil-to-lymphocyte ratio, solid tumors, palliative chemotherapy, best supportive care

Öz

Amaç: Bu çalışmada nötrofil-lenfosit oranının (NLO), metastaik kanser hastaları için palyatif kemoterapiye devam etme veya en iyi destekleyici bakımı (BSC) seçme konusunda bir belirleyici olup olmadığını araştırmayı amaçladık.
Yöntemler: Palyatif kemoterapi sonrası ölen metastatik kanser tanılı hastalar çalışmaya dahil edildi. Hastalar son kemoterapi rejimlerinin başlangıcı ile ölüm (DCD) arasındaki süreye göre ≤ 60 veya > 60 güne göre iki gruba ayrıldı. Nötrofil-lenfosit oranı, son kemoterapi hattı başlangıcından öncesindeki laboratuvar değerleri kullanılarak hesaplandı. ≤ 60 günlük DCD'nin belirleyici faktörleri lojistik regresyon analizi ile incelendi ve istatistiksel anlamlılık düzeyi alfa $p < 0,05$ olarak kabul edildi.
Bulgular: Çalışmaya ortalama tanı yaşı 61,7 ± 12,0 yıl olan 404 hasta dahil edildi. Ortalama NLO 11,3 ± 27,1 olarak hesaplandı. ≤ 60 günlük DCD belirlenmesi için tek değişkenli analizde, meme ve kolorektal kanser tanıları, ECOG durumu, tek ajan kemoterapi kullanımı, nötrofil sayısı, lenfosit sayısı ve NLO önemli faktörler olarak bulundu. ≤ 60 günlük DCD'yi belirleyen kesim değeri NLO ≥ 3,59 olarak belirlendi. Lojistik regresyon analizinde, NLR ≥ 3,59 ve ECOG durumu önemli faktörler olarak bulundu.
Sonuç: ECOG performans durumu ile kombine edilmiş nötrofil-lenfosit oranı, metastatik kanser hastalarında sağkalımı tahmin edebilir ve bu nedenle klinisyenlerin hastalarına kemoterapi vermeyi veya onları en iyi destekleyici bakıma yönlendirmeyi seçmelerine yardımcı olabilir.

Anahtar Kelimeler: Nötrofil-lenfosit oranı, solid tümörler, palyatif kemoterapi, en iyi destekleyici bakımı

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## Introduction

The short- and long-term prognoses of cancer depend on patient and tumor features such as age, performance status, tumor site, grade, stage and treatment modality [1]. The tumor microenvironment and, in particular, the inflammatory response are thought to play important roles in cancer development and progression, and may be associated with systemic inflammation [2]. The neutrophil-to-lymphocyte ratio (NLR) is a novel marker of inflammation and is measured through routine blood count tests. It becomes elevated in metabolic and inflammatory conditions that are associated with chronic low-grade inflammation. These conditions include diabetes mellitus, thyroiditis, obesity and ulcerative colitis. NLR is even correlated with HbA1c levels in diabetic patients. Moreover, it helps in differentiating malignant nodules from benign ones in thyroid glands [3-8]. NLR has also been linked to a variety of malignancies such as lung, esophageal, colorectal, ovarian, and head and neck cancers [9-13].

For medical oncologists, determining a prognosis and life expectancy is critical to choosing either best supportive care (BSC) or chemotherapy. Survival estimates that clinicians make, usually guided only by their intuition and clinical experience, are often incorrect, and clinicians tend to believe that their patients have longer to live than they actually do [14]. This error sometimes results in treatment that is too aggressive [15, 16]. Although physicians appear to be wrong less often when assessing short- (<15 days) and long-term (>6 months) survival, there is a substantial period of uncertainty— a better prognostic assessment could help improve patient care [15]. While prognostic factors and predictive tools have been explored and developed to improve a clinician's ability to estimate life expectancy, they often require complex parameters, such as the inclusion of patient and tumor features [17, 18].

We investigated the NLR's ability to act as a predictor in deciding whether to continue palliative chemotherapy or to instead employ BSC in advanced cancer patients.

## Material and methods

This trial was planned as a retrospective single-center study. Medical details were obtained from the archive files of patients with advanced solid tumors, who had died between January 2018 and December 2019 after palliative chemotherapy treatment in the medical oncology clinic of Prof. Dr. Cemil Taşçıoğlu City Hospital. These were patients who had been admitted to the oncology clinic and would routinely, after a 12-hour fast, have blood samples taken. The blood was drawn from the antecubital vein and a blood analysis was performed. Tubes containing ethylenediaminetetraacetate (for complete blood counts) and anticoagulant-free gel tubes (for biochemical parameters) were used to store the blood samples. The complete blood count parameters were tested in a hemogram autoanalyzer (Mindray, China), and the biochemical parameters were examined in an autoanalyzer (Beckman Coulter, USA), using a colorimetric method. Disease staging was performed according to the Tumor, Node, Metastasis (TNM) staging system. Patients with missing data were not included in the study. Patients with infectious diseases, other inflammatory diseases such as rheumatoid arthritis and ulcerative colitis, hematologic malignancies, and patients who had received granulocyte colony-stimulating factor ≤ 4 weeks before last chemotherapy line were excluded from the study. The patients were divided into two groups, according to DCD, as ≤ 60 or > 60 days [15].

The demographic features included age at diagnosis and death, histologic type, Eastern Cooperative Oncology Group (ECOG), status both at diagnosis and before beginning the last chemotherapy regimen, as well as stage, the number of total treatment lines, the last chemotherapy modality (single agent or combination) and the time between the beginning of their last chemotherapy regimen and death (DCD). Also noted were the laboratory values before the beginning of the last chemotherapy line, such as white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), mean platelet volume (MPV), total platelet count (TPC), total neutrophil count (TNC), total lymphocyte count (TLC), total monocyte count (TMC), creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH), and C reactive protein (CRP). The NLR was calculated by dividing the TNC count by TLC. Biochemical parameters were presented as either below or above the reference range, based on the reference intervals used in the laboratory.

**Statistical Analysis**

SPSS 15.0 for Windows was used for statistical analysis. Descriptive statistics were given as a number and as a percentage for categorical variables, average and standard deviation, and as a minimum and maximum for numeric variables. Comparisons of the numerical variables in two independent groups were made using the Mann Whitney U test, since the normal distribution condition was not met. Comparisons of the ratios in the groups were made using the Chi-Square test. The determinant factors which p value < 0.250 in univariate analysis, were examined by logistic regression analysis, the cutoff value was calculated by ROC curve analysis and a statistical significance level of alpha was accepted as $p < 0.05$.

## Results

The study included 404 patients (68.8% men and 31.2% women) with solid tumors who had died after palliative chemotherapy. The mean age at diagnosis was 61.7±12.0 (22–89) years. The five most common cancer types were non-small-cell lung (33.4%), gastric (15.3%), small-cell lung (9.9%), colorectal (8.7%) and breast cancers (6.9%). The patient numbers for local, locally advanced and metastatic stage at the diagnosis were 22 (5.4%), 97 (24.0%) and 285 (70.5%), respectively. The mean number of chemotherapy lines was 1.40 (min-max 1–8). The number of patients that received single, doublet and triplet chemotherapy as a last chemotherapy regimen was 133 (32.9%), 228 (56.4%) and 43 (10.6%), respectively. The number of patients with an ECOG status of 0, 1, 2 and 3 before beginning the last chemotherapy regimen was 10 (2.5%), 40 (9.9%), 316 (78.2%) and 38 (9.4%), respectively. The mean DCD was 60.6±99.9 days (0–962). The number of patients in the two groups according to the DCD, whether ≤ 60 or > 60 days, was 291 (72.0%) and 113 (28.0%), respectively (Table 1).

The mean TNC and TLC counts were 7.32±5.52*103uL and 1.29±0.87*103uL, respectively. The mean NLR was calculated as 11.3%±27.1%. Other laboratory parameters are laid out in Table 2.

In the univariate analysis, breast and colorectal cancers were higher in patients who lived ≤ 60 days after the last chemotherapy than in those who lived > 60 days ($p = 0.035$ and $p = 0.040$, respectively). Also, single-agent chemotherapy usage was higher in the ≤ 60 days-group. There was a significant difference between the groups with respect to ECOG status. The number of ECOG 0/1 patients was higher in > 60-days group, and the ECOG 2/3 patient number was higher in the ≤ 60-day group ($p = 0.002$) (Table 1). The mean TNC was higher (7.75±5.76 103uL and 6.21±4.69 103uL, respectively, where $p = 0.014$) and the TLC was lower (1,22±0.87 103uL and 1,48±0.83 103uL, respectively, where $p = 0.003$) in the ≤ 60-days group

than in > 60-days group.

Table 1. Demographic and pathologic features of patients and univariate analysis for determining the 60≤days survival.

| Variables | | All patients (n= 404) | | 60≤days (n=291) | | >60 days (n=113) | | p |
|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % | |
| Gender | Male | 278 | 68.8 | 202 | 69.4 | 76 | 67.3 | 0.674 |
| | Female | 126 | 31.2 | 89 | 30.6 | 37 | 32.7 | |
| Age at diagnosis(year) | Mean+SD | 61.7±12.0 (22-89) | | 62.0±12.2 | | 60.8±11.8 | | 0.350 |
| Diagnosis | NSCLC | 135 | 33.4 | 98 | 33.7 | 37 | 32.7 | 0.858 |
| | SCLC | 40 | 9.9 | 33 | 11.3 | 7 | 6.2 | 0.120 |
| | Breast | 28 | 6.9 | 25 | 8.6 | 3 | 2.7 | 0.035 |
| | Colorectal | 35 | 8.7 | 20 | 6.9 | 15 | 13.3 | 0.040 |
| | Prostate | 7 | 1.7 | 4 | 1.4 | 3 | 2.7 | 0.405 |
| | Gastric | 62 | 15.3 | 41 | 14.1 | 21 | 18.6 | 0.261 |
| | RCC | 1 | 0.2 | 1 | 0.3 | 0 | 0.0 | 1.000 |
| | Sarcoma | 8 | 2.0 | 6 | 2.1 | 2 | 1.8 | 1.000 |
| | Pancreas | 20 | 5.0 | 16 | 5.5 | 4 | 3.5 | 0.415 |
| | Bladder | 11 | 2.7 | 5 | 1.7 | 6 | 5.3 | 0.081 |
| | Other | 57 | 14.1 | 42 | 14.4 | 15 | 13.3 | 0.764 |
| Last Ctx regimen | Single | 133 | 32.9 | 109 | 37.4 | 26 | 23.0 | 0.024 |
| | Doublet | 228 | 56.4 | 157 | 54.0 | 72 | 63.7 | |
| | Triplet | 43 | 10.6 | 25 | 8.6 | 15 | 13.3 | |
| Chemotherapy line number | Med (min-max) | 2 (1-8) | | 2 (1-8) | | 1 (1-7) | | 0.684 |
| ECOG before last Ctx regimen | 0 | 10 | 2.4 | 6 | 2.1 | 4 | 3.6 | 0.002 |
| | 1 | 40 | 10.0 | 19 | 6.6 | 21 | 18.8 | |
| | 2 | 316 | 78.2 | 235 | 81.0 | 79 | 70.5 | |
| | 3 | 38 | 9.4 | 30 | 10.3 | 8 | 7.1 | |
| DCD (days) | Mean+SD | 60.6± 99.9 | | | | | | |

ECOG: Eastern Cooperative Oncology Group scales, Ctx: chemotherapy, NSCLC: non-smallcelllungcancer, SCLC: small cell lungcancer, RCC: renal cell carcinoma, DCD: duration between last chemotherapy regimen and death, min: minimum, max: maximum, SD: standard deviation

The mean NLR values were 13.5±31.4% and 5.8±6.5%, respectively, in ≤ 60 days group and in > 60-days group ($p < 0.001$). Also, AST (51.1±85.8 and 39,2±89.6, respectively, where $p = 0.004$) and ALT values (36.7±45.2 and 27.0±63.2, respectively, where $p < 0.001$) were higher in the ≤ 60-days group than in > 60-days group. There was no difference in terms of CRP and LDH between the groups. (Table 2)

Table 2. Laboratory features of patients and univariate analysis for determining the 60≤days duration between last chemotherapy regimen and death

| Variables | Allpatients (n=404) mean±SD | 60≤ days (n=291) mean±SD | >60 days (n=113) mean±SD | p |
|---|---|---|---|---|
| WBC (10^3/uL) | 9.36±5.99 | 9.69±6.22 | 8.52±5.30 | 0.146 |
| Neu (10^3/uL) | 7.32±5.52 | 7.75±5.76 | 6.21±4.69 | 0.014 |
| Lym (10^3/uL) | 1.29±0.87 | 1.22±0.87 | 1.48±0.83 | 0.003 |
| NLR % | 11.3±27.1 | 13.5±31.4 | 5.8±6.5 | <0.001 |
| Eos (10^3/uL) | 0.11±0.27 | 0.10±0.3 | 0.14±0.24 | 0.019 |
| Hgb (g/dL) | 10.8±1.7 | 10.9±1.7 | 10.6±1.6 | 0.212 |
| Plt (10^3/uL) | 271.4±161.3 | 265.4±158.1 | 286.8±169.0 | 0.292 |
| PDW (fL) | 14.3±8.1 | 14.2±2.5 | 14.6±14.8 | 0.004 |
| Crea (mg/dL) | 0.87±0.58 | 0.85±0.57 | 0.90±0.60 | 0.205 |
| | Median (min-max) | Median (min-max) | Median (min-max) | |
| AST (U/L) | 24 (6-920) | 26 (6-920) | 20 (7-917) | 0.004 |
| ALT (U/L) | 20 (3-645) | 23 (3-411) | 17 (5-645) | <0.001 |
| LDH (U/L) | 238 (22-4552) | 237 (23-4478) | 239 (22-4552) | 0.987 |
| CRP (mg/dL) | 32 (0.75-1427) | 34 (0.75-1427) | 25 (1.92-375) | 0.330 |

WBC: white blood count, Neu: neutrophil, Lym: lymphocyte, NLR: neutrophil to lymphocyte ratio, Eos: eosinophil, Hgb: hemoglobin, Plt: platelet, PDW: platelet distribution width, Crea: creatinine, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, SD: Standard deviation

The receiver operating characteristic (ROC) curve was drawn using the NLR ratios at the time before the beginning of the last chemotherapy line; the corresponding area under the curve value was found to be 0.636 (95% Cl 0.577–0.694, $p < 0.001$). The cutoff value determining the ≤ 60-day DCD was determined as NLR ≥ 3.59%, with 70.0% sensitivity and 51.0% specificity (Fig. 1). The median DCD was lower in patients with NLR ≥ 3.59% than <3.59% (26 days and 42 days, respectively, where p= 0.001) (Table 3).

Table 3. Survivals differences between groups according to NLR cut-off value.

| | NLR | | | | |
|---|---|---|---|---|---|
| | <3.59 | | ≥3.59 | | |
| | Mean ±SD | Min-Max (Median) | Mean ±SD | Min-Max (Median) | p |
| DDD (month) | 13.8±20.6 | 0-150 (7) | 9.4±14.5 | 0-148 (5) | 0.002 |
| DCD (day) | 86.4±130.0 | 2-810 (42) | 47.0±76.2 | 0-962 (26) | 0.001 |

DDD: duration between diagnosis and death, DCD: duration between last chemotherapy regimen and death, NLR: neutrophil to lymphocyte ratio, Min: minimum, Max: maximum, SD: Standard deviation.

In multivariate logistic regression analysis for factors determining the ≤ 60-day DCD, an NLR ≥ 3.59%, as well as ECOG status, were found to be significant factors ($p < 0.001$, and $p < 0.009$, respectively) (Table 4).

Table 4. Multivariate analysis for determining the 60≤days duration between last chemotherapy regimen and death.

| | | p | OR | %95 CI | |
|---|---|---|---|---|---|
| ECOGs at before last ctx regimen | | 0.003 | | | |
| | 0 | 0.631 | 0.662 | 0.123 | 3.564 |
| | 1 | 0.009 | 0.225 | 0.074 | 0.685 |
| | 2 | 0.819 | 0.899 | 0.363 | 2.230 |
| Stage at diagnosis | | 0.875 | | | |
| | 2 | 0.989 | 0.992 | 0.290 | 3.396 |
| | 3 | 0.787 | 1.211 | 0.301 | 4.869 |
| Chemotherapy line number | | 0.627 | 1.106 | 0.737 | 1.658 |
| WBC | | 0.902 | 1.003 | 0.956 | 1.052 |
| NLR | ≥3.59 | <0.001 | 2.696 | 1.553 | 4.679 |
| Hgb | | 0.419 | 1.068 | 0.911 | 1.252 |
| MPV | | 0.722 | 0.963 | 0.785 | 1.183 |
| Crea | | 0.484 | 0.864 | 0.574 | 1.300 |
| ALT | | 0.167 | 1.004 | 0.998 | 1.009 |
| Diagnosis | SCLC | 0.511 | 1.382 | 0.527 | 3.626 |
| | Breast | 0.058 | 4.693 | 1.086 | 20.287 |
| | Colorectal | 0.251 | 0.616 | 0.270 | 1.409 |
| | Bladder | 0.525 | 0.620 | 0.142 | 2.710 |

ECOG: Eastern Cooperative Oncology Group scales, WBC: white blood count, NLR: neutrophil to lymphocyte ratio, Hgb: hemoglobin, MPV: mean platelet volume, Crea: creatinine, ALT: alanine aminotransferase, Ctx: chemotherapy, SCLC: small cell lung cancer,

## Discussion

In this study, our aim was to investigate whether NLR is a predictor of survival in cancer patients that received palliative chemotherapy. We found that NLR and ECOG status were independent factors for ≤ 60 days' survival in patients with advanced solid tumors.

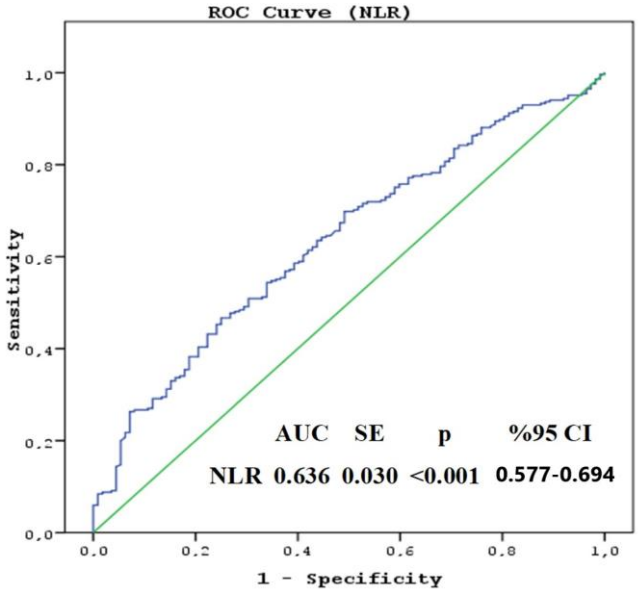


Fig. 1: Receiver operating characteristic curve analyses for ≤ 60day survival.
The cutoff value determining the ≤ 60 day DCD, was determined as NLR ≥ 3.59%, with 70.0% sensitivity and 51.0% specificity.

NLR is an indicator of inflammation and immune system response, and has been accepted as a prognostic factor in various malignancies. Based on the findings of current studies, it is relatively consistent to conclude that a higher NLR is a negative prognostic factor for many cancer types such as renal cell carcinoma, malignant melanoma, and gastric, pancreatic, breast and colorectal cancers [19-28]. But a few studies have focused on which parameter is a predictor of early death. In a trial published in 2007, with a total of 177 patients with solid tumors, two-month survival predictors were investigated, and the Karnofsky index, the number of metastatic sites, low serum albumin and LDH concentration were found to be independent factors in predicting two months' survival. Also, with univariate analysis, a high WBC was found to be a poor prognostic factor, but this relation was not observed with multivariate analysis. The neutrophil-to-lymphocyte ratio was not evaluated in this study [15].

Earlier studies have focused solely on performance status in terms of survival estimation. For example, one study set out to improve the ability to estimate the survival of terminally ill cancer patients and found that the factor most strongly associated with shorter survival was poor performance status [1]. However, these patients' NLR was not included in the analysis. Also, a palliative performance scale (consisting of the subheadings of mobility, activity/disease finding, self-care, nutrition and level of consciousness) was evaluated for estimating survival. The study's findings revealed that the palliative performance scale upon admission, along with gender and age, was a strong predictor of survival in patients already identified as palliative. However, survival had not been a significant part of the diagnoses [29]. A trial published in 2008 set out to derive and validate a simple predictive model for the survival of patients who had metastatic cancer and attended a palliative radiotherapy clinic. This model, different from older models (including six separate factors) needed three prognostic factors: primary cancer site, site of metastases and KPS. The study did not find a difference between the two models in terms of estimating survival [30]. In another study, which included a total of 299 patients, the prognostic value was based on a combination of performance status (PS) factors, with either the LDH level or the lymphocyte count being evaluated. This study found that a PS > 1, a lymphocyte count ≤ 700/μL and LDH > 600 UI/L were independent predictors of short-term survival, as well as the interleukin 6 (IL-6) level, the serum albumin concentration and the platelet count [31]. In the studies discussed above, the researchers either focused only on the PS or on models with complex components, but they did not evaluate NLR in terms of survival estimation. We found that easily accessible parameters such as NLR and ECOG status were independent factors for ≤ 2 months' survival in patients with advanced solid tumors. Also, we found that in the study's population, there was no relation between LDH and a ≤ two-months survival time.

This is a retrospective study with a specific limitation: We could not include albumin values in the analysis since not all patients had the respective data. But this is the first study to focus on the NLR as a predictor in terms of survival estimation in patients with advanced solid tumors, independent of tumor type.

In conclusion, NLR combined with ECOG PS appears to better predict survival in patients with solid advanced tumors and thereby can help clinicians either administer chemotherapy to their patients or direct them to the best supportive care.

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# Small bowel transplantation management during COVID-19 pandemic in a child with microvillus inclusion disease from Turkey

## Türkiye'den mikrovillus inklüzyon hastalığı olan bir çocukta COVID-19 pandemisi sırasında ince bağırsak nakli yönetimi

**Maşallah Baran [1], Yeliz Çağan Appak [1], Betül Aksoy [2], İsmail Sert [3], Onur Işık [4], Göksever Akpınar [3], M.Onur Öztan [5], Muhammet Akyüz [4], Ayşe Berna Anıl [6], Eyüp Kebapçı [3], Tülay Kılıçaslan Ayna [7], Yalçın Güvenli [8], Cem Tuğmen [3]**

Abstract

Microvillus inclusion disease is a cause of congenital intestinal intractable diarrhea. Total parenteral nutrition is required throughout life and the patients need intestinal transplantation for survival. The new Coronavirus Disease 2019 (COVID-19) caused a worldwide pandemic in January 2020. Emergency surgical interventions are suggested to carrying out by taking appropriate measures during the pandemic period. In this case report, we aimed to discuss the small bowel transplantation management during the COVID-19 pandemic by presenting a child with microvillus inclusion disease who was underwent emergency small bowel transplantation due to the appropriate cadaveric organ donation.

Key words: The new Coronavirus Disease 2019, small bowel transplantation, microvillus inclusion disease, child

Öz

Mikrovillus inklüzyon hastalığı, konjenital intestinal inatçı diyare nedenidir. Yaşam boyunca total parenteral beslenme gerekir ve hastaların sağkalım için bağırsak nakline ihtiyacı vardır. Yeni Coronavirus Hastalığı 2019 (COVID-19), Ocak 2020'de dünya çapında bir pandemiye neden oldu. Acil cerrahi müdahalelerin pandemi döneminde uygun önlemler alınarak yapılması önerilmektedir. Bu olgu sunumunda, uygun kadavra organ bağışı nedeniyle acil ince bağırsak nakli yapılan mikrovillus inklüzyon hastalığı olan bir çocuğu sunarak COVID-19 salgını sırasında ince bağırsak nakli yönetimini tartışmayı amaçladık.

Anahtar kelimeler: Yeni Koronavirüs Hastalığı 2019, ince bağırsak nakli, mikrovillus inklüzyon hastalığı, çocuk

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## Introduction

Microvillus inclusion disease (MID) is a known congenital cause of intractable diarrhea resulting intestinal failure that need intestinal transplantation for survival. Novel Coronavirus Disease 2019 (COVID-19), which was first identified in Wuhan city of China in December 2019, and is the cause of pandemic worldwide in January 2020. SARS-CoV-2 (COVID-19 virus) was identified as the responsible agent. The first cases were identified in Turkey in March 2020 [1]. During the pandemic period, only emergency surgical interventions were carrying out by taking appropriate measures. To the best of our knowledge, mostly liver, kidney, lung and heart transplantation have been reported from solid organ transplantations during the COVID-19 pandemic period, there is no small bowel transplantation (SBTx) notification [2]. We present a case with MID who underwent SBTx during the COVID-19 pandemic due to the appropriate cadaveric organ donation that he had been waiting for one year.

## Case report

An 18-month-old boy who had intractable diarrhea since the neonatal period, and was diagnosed MID, was being followed up in our center for SBTx for one year. He was receiving total parenteral nutrition (TPN) support, and he had enteral feeding intolerance. In genetic analysis, c.4399C> T homozygous was detected in the MYOB5B gene. SBTx was planned, but he waited eight months in cadaveric transplantation list for appropriate donor. With the onset of the COVID-19 pandemic period, the patient was started to follow in the organ transplant service with being pay attention to all isolation and protective measures. While the first month of COVID-19 pandemic in our country, organ donation was performed compatible for the patient's age-weight and tissue-type. The SARS CoV-2 polymerase chain reaction (PCR) analysis was negative, the patient had no fever and lung findings. He had no signs of infection and transplant preparations were initiated. The entire transplant team worked in accordance with the mask and hygiene rules pre-operation and during the operation process. Before the operation, SARS CoV-2 PCR was applied to the donor and it was found to be negative. Donor was a 2 years old girl deceased due to trauma. Before the operation, the family was informed about the risks of surgical intervention during the COVID-19 period, and a detailed consent form was obtained. Isolated small bowel segment was transplanted to our case. Anti-thymocyte globulin (ATG) and high dose steroid regimen were used in induction period. No additional treatment regimen was applied for COVID-19. During this period, low dose ganciclovir treatment and was broad-spectrum antibiotherapy was used according to our transplantation protocol. Post-transplant follow-up of the patient was performed in our pediatric intensive care clinic, where there was no patient with COVID-19. In this isolated area, healthcare staff wasn't following the case with COVID-19 disease. In the postoperative follow-up, all healthcare personnel paid attention to the contact and isolation measures of the patient who was under immunosuppression. After twelve days, the patient was taken to the organ transplant service and the caregiver was evaluated with SARS CoV-2 PCR. After the test was found to be negative, the caregiver was allowed to take care of the patient. The patient was taken to intensive care unit due to septic shock after four days, and his SARS CoV-2 PCR negative. Ostomia output was increased, and fecal rotavirus test was positive. Oral immunoglobulin regimen was used. Donor specific anticor test was detected negative. Graft was found to be minimally edematous in endoscopy that was performed according to COVID-19 measures. Acute severe rejection was developed at the 20th day after transplantation. Rituximabe was started following ATG and pulse steroid regimen for rejection, after SARS CoV-2 PCR was detected negative. But there was no response to rejection treatment. The graft had to be removed due to severe rejection one month after SBTx.

Written informed consent was obtained from parent for publication.

## Discussion

In our patient who underwent SBTx during the COVID-19 pandemic, sepsis and rejection were seen. Sepsis and rejection are the most common complications after SBTx. It is seen that COVID-19 has no effect on morbidity in this case. After this operation, which requires immunosuppressive treatment, the patient was protected by paying attention to all COVID-19 precautions during pre-operation, intra-operation and post-operation term.

Unfortunately, organ donation in childhood is not sufficient in our country. Therefore, patients who are candidate for SBTx, can wait for a very long time in cadaver waiting list [3]. Therefore, emergency transplantation conditions are provided when a suitable donor is detected. Nevertheless, the effects of perioperative physiological stress on predisposition to or recovery from COVID-19 are not known but it is assumed that relative immunocompromise after major surgical intervention worsens the prognosis of those who either contract COVID-19 perioperatively or have been asymptomatic carriers prior to intervention. Infection with this virus can complicate the perioperative course and prove a significant diagnostic challenge with an unacceptably high fatality rate [4]. Patient-to-patient, and patient-to-healthcare worker infection was described and human-to-human transmission has been confirmed. As such, strict infection prevention practices are essential [5].

According to the guideline of the Ministry of Health in our country, it is recommended to carry out all kinds of medical care and interventions that are indicated by taking necessary and appropriate measures during the COVID-19 pandemic period. It is recommended to apply the use of personal protective equipment (PPE) for all patients during the operation. Necessary PPEs in the operating room: N95 / FFP2 mask, sterile surgical gown, disposable sterile gloves, goggles / face protector, disposable bone, disposable foot protector / shoe covers, alcohol-based hand antiseptic are recommended. It is recommended to perform normal surgical procedures in patients who have not COVID-19 infection. If the patient has a diagnosis or suspicion of COVID-19, non-emergency surgeries should canceled or postponed. Anesthesia and surgical intervention may adversely affect the course of COVID-19. It is recommended that the patient and her family be informed about the increased risk of COVID-19 related surgery in emergency surgeries and that they give approval with a detailed consent form [6]. During the operation, we performed the transplantation by paying attention to all these PPEs and obtaining a consent form from the family.

According to the recommendations of The Transplantation Society; persons who have been exposed to a patient with confirmed or suspected COVID-19 within 14 days should not be accepted as a donor. Likewise donors with unexplained respiratory failure leading to death should be excluded. Donors with positive PCR testing for COVID-19 should not be utilized [7]. Some national guidelines recommend routine testing of donors for SARS-CoV-2. Combining epidemiological data and PCR testing is one approach that has been used. Additionally, in a country with widespread community transmission, temporary suspension of the deceased

donor program should be considered, especially when resources at the transplant center may be constrained [8]. If a transplant candidate is sick and found to be infected with COVID-19, transplant should be deferred until clinically improved with no detectable virus. Prolonged viral shedding has been described. Documentation of negative PCR testing at least 24 hours apart is recommended before a candidate should be cleared for transplant unless the need for transplant is urgent. Ideally, patients should be tested 10-14 days after symptom onset and only once symptoms have resolved. Patients should have 2 negative PCR tests done at least 24 hours apart [9].

In a study evaluating liver transplants in pandemic period in Italy, it is reported that donors and recipients were screened for SARS-CoV-2. In the postoperative period, two of the 17 LT patients had positive SARS CoV-2 PCR test and one patient died due to Covid-19. They suggested that only patients with true end-stage liver disease and extremely poor prognosis should undergo LT [10]. Changes in immunosuppression treatment are also not well studied in the transplant populations. Calibration of dose reduction has to balance consequences of rejection. We did not reduce the immunosuppression treatment post-transplant period in our patient, too.

Consequently, emergency solid organ transplants can be carried out during COVID-19 period. The important thing is to take protective measures that can ensure the safety of the recipient and the healthcare staff. It is important for the recipient to be maintained protective measures under immunosuppressive treatment before and after transplantation in order to increase survival. We used this option in the patient who had no chance of survival other than SBTx. However, the rejection was developed independent of the COVID-19 pandemic. For this reason, if the conditions of the transplant clinic are appropriate during the pandemic period, we believe that the SBTx program is sustainable for emergent and selected cases after all measures are taken.

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# Breast conservation with batwing mastopexy for the management of giant juvenile fibroadenoma: A case report of a 12-year-old girl

## Dev juvenil fibroadenomun yönetiminde batwing mastopeksi yöntemi ile meme koruyucu cerrahi: 12 yaşında bir kız çocuk olgu sunumu

**Bülent Çitgez [1], Elif Baran [1], Banu Yiğit [1], Soysal Baş [2], Aydın Eray Tufan [1], Hamdi Özşahin [1]**

Abstract

Fibroadenomas are benign breast tumours consisting of epithelial and stromal components. Most of them are about 1 to 2 cm in size. It is defined as “giant” when the fibroadenoma is larger than 5cm, weighs more than 500 g or occupies for at least 80% of breast volume. It is usually encountered in patients of less than 20 years of age. Progressively growing mass and its large size cause a suspicion of malignancy. It presents as unilateral macromastia that causes breast asymmetry. Excellent cosmetic results cannot be obtained with simple excision in this situation. We aimed to present a case of a 12-year-old patient with a diagnosis of giant juvenile fibroadenoma. In this case, we performed surgical excision with Batwing mastopexy of the breast to achieve optimal symmetry with contralateral breast.

Key words: Batwing, fibroadenoma, mastopexy, breast, symmetry

Öz

Fibroadenomlar, epitel ve stromal bileşenlerden oluşan iyi huylu meme tümörleridir. Çoğu yaklaşık 1 ila 2 cm boyutlarındadır. Fibroadenom 5 cm'den büyükse, 500 g'dan fazla ağırlığa sahipse veya meme hacminin en az % 80'ini kapladığında "dev" olarak tanımlanır. Genellikle 20 yaş altı hastalarda görülür. Kitlenin progresif olarak büyümesi ve boyutları, malignite şüphesine neden olur. Genellikle meme asimetrisine neden olan tek taraflı makromasti olarak kendini gösterir. Bu durumda basit eksizyon ile mükemmel kozmetik sonuçlar elde edilemeyebilir. Dev juvenil fibroadenom tanısı alan 12 yaşında bir hastayı sunmaktayız. Bu durumda, kontralateral meme ile optimal simetri elde etmek için Batwing mastopeksi tekniği ile cerrahi eksizyon yaptık.

Anahtar kelimeler: Batwing, fibroadenom, mastopeksi, meme, simetri

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## Introduction

Fibroadenomas are benign breast tumours consisting of epithelial and stromal elements and occur in approximately 10 percent of women [1]. The exact etiology and pathogenesis underlying fibroadenomas is not clearly understood. It has been reported that it can be associated with the use of hormonal contraceptives or detection of Epstein-Barr virus [2]. Most of them are about 1 to 2cm in size. Growing rapid of fibroadenomas usually slows down when they reach 2 to 3cm in size [3]. It is defined as "giant" when the fibroadenoma is larger than 5cm, weighs more than 500 g or occupies for at least 80% of breast volume. Giant fibroadenoma of the breast usually occurs in patients younger than 20 years of age [4, 5]. Fibroadenomas are ought to be benign lesions but they have tendency to grow faster. This feature may suggest the possibility of malignancy and it may need a comprehensive differential diagnosis. The epithelial component of fibroadenoma may be the origin of the malignant tumors especially lobular carcinoma. Unilateral fibroadenomas occur with a serious breast asymmetry due to their rapid enlargement; therefore they cannot be improved with just a simple surgical excision. Batwing mastopexy is an oncoplastic technique for periareolar lesions in the upper central breast and especially for lesions in pitotic breasts [6]. This method also can be applied in the treatment of giant fibroadenomas.

## Case report

A 12-year-old female patient referred to the surgical department of our hospital with a complaint of fast-growing, painful mass of her right breast that she first noticed 6 months ago. Patient reported that her menarche onset age was 11 and stated that her menstrual cycle pattern was also normal. The patient has no family history of breast disease or family history for cancer.

Physical examination, showed a palpable mass larger than 10cm in upper outer quadrant of the right breast. The mass was almost completely filling the right breast. The mass caused the unilateral enlargement of the right breast and asymmetrical appearance compared to her left side (Figure1). Clinical examination of the left breast was normal and there was no axillary lymphadenopathy.

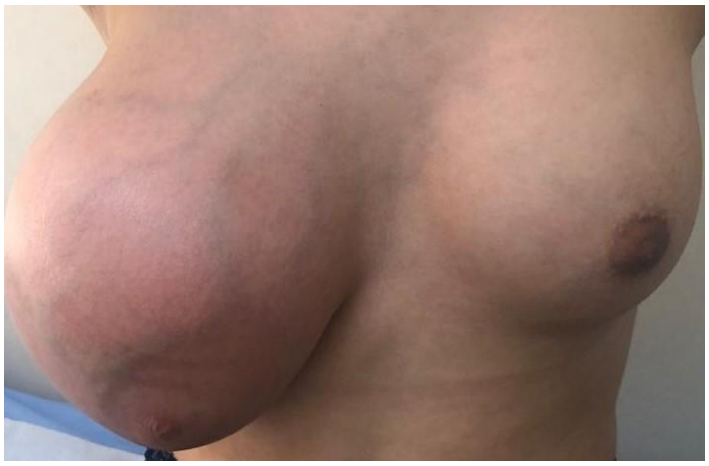

Figure 1: Enlarged right breast due to a giant juvenile fibroadenoma mass.

Breast ultrasonography was performed via high frequency (6-11 MHz) linear transducer. Breast ultrasound revealed a homogeneous, hypoechoic, solid mass in upper outer quadrant of the right breast with a diameter of 134x91mm that filled the breast almost completely. No axillary nodes were noticed in the bilateral axilla. Phyllodes tumors, juvenile breast hypertrophy, giant lipomas and hamartomas were considered for differential diagnosis. Ultrasound guided core needle biopsy (CNB) was performed and the biopsy result was reported to be compatible with pseudoangiomatous stromal hyperplasia. Due to clinical suspicion and cosmetic reasons, the patient was subjected to surgical intervention. Surgical excision was performed using the Batwing mastopexy technique. The postoperative follow-up was uneventful; the patient had excellent cosmetic results. Breasts were a in perfect symmetry and the right breast matched to the size and shape of the other breast (Figure 2). The macroscopic assessment of pathology specimens showed homogeneous cut surface and encapsulated lobular contour with a tumour size of 22x18x7cm (Figure 3, 4). The excised mass weighed 1600g. After surgical excision, histopathology confirmed the diagnosis of giant juvenile fibroadenoma with pseudoangiomatous stromal hyperplasia in the focal areas. Atypia or pleomorphism was not observed.

Written informed consent was obtained from parent for publication.

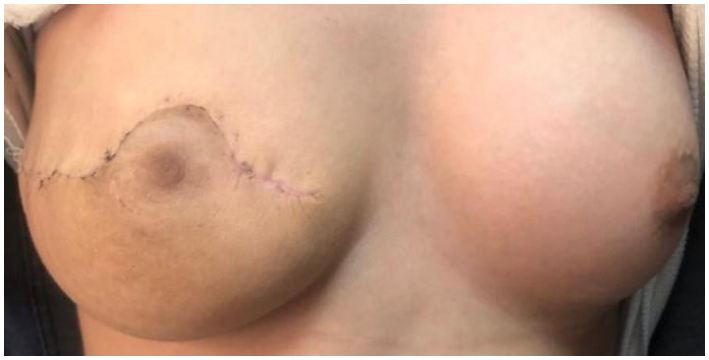

Figure 2: 1 month after fibroadenoma excision and breast reconstruction with Batwing mastopexy.

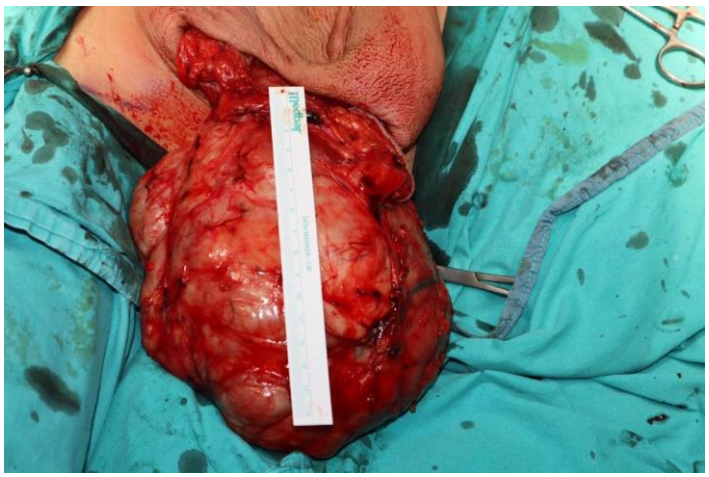

Figure 3: Macroscopic view of the excised giant fibroadenoma with well circumscribed lobulated contours.

## Discussion

We report a case with juvenile giant fibroadenoma underwent a wide excision of the tumor in a 12-year old girl. Fibroadenomas are called "juvenile fibroadenoma" when the patient is under 18 years of age and usually encountered during the adolescent period [7]. The prevalence of fibroadenoma during the adolescent period is 2.2% and 1-8% of fibroadenomas are giant juvenile fibroadenomas [8, 9]. "Giant" fibroadenomas are called fibroadenomas larger than 5cm or heavier than 500grams or when the mass is greater than 80% of the normal

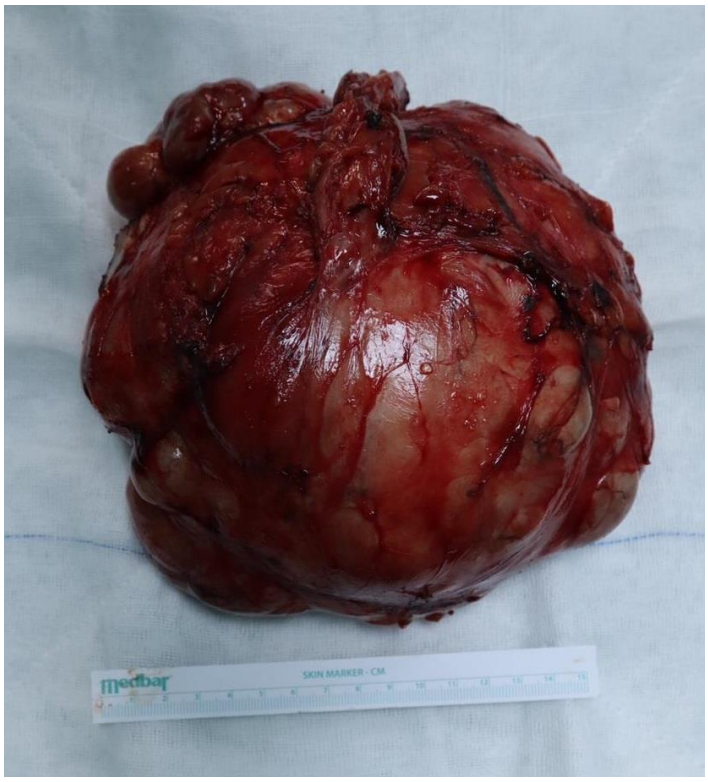

Figure 4: Macroscopic view of the excised giant fibroadenoma with well circumscribed lobulated contours.

breast size [4]. In our case, fibroadenoma was complex, larger than 5 cm (22cm) and its weight was heavier than 500 g (1600 g). Histologically fibroadenomas divided into two groups which are simple and complex. Most of them are benign breast lesions, but the risk of malignancy increases in complex fibroadenomas. Complex fibroadenomas are differentiated from simple ones by containing different cell types [10]. Etiological factors have not been exactly known, but reproductive hormones or history of previous breast traumas are thought to be the reasons [11, 12].

Juvenile fibroadenomas are usually presented as palpable masses of the breast that are hard and movable lumps with well-defined margins. They are painless masses when they are small in size. They may become tender or painful as they increase in size or before menstrual period. In our case, she presented with a painful and rapidly enlarging unilateral breast mass. Fibroadenomas can cause breast asymmetry, nipple retraction, atrophy of surrounding breast parenchymal tissue and widened superficial blood vessels. Giant juvenile fibroadenomas may mimic malignant breast tumours because of their rapid growth. Whereas malignant transformation is rare in fibroadenomas; phyllodes tumors and adenocarcinomas should be evaluated in differential diagnosis [13, 14].

Ultrasonography is an important diagnostic method for the diagnosis of fibroadenoma and detecting masses in fibroglandular breasts. If it is required, imaging studies such as mammography or magnetic resonance imaging studies should also be performed. In our case, ultrasound was performed as the first imaging method in accordance with the age and the breast tissue density of the patient. Fine needle aspiration biopsy (FNAB) and CNB can be used in diagnosis of the suspected fibroadenoma [15, 16]. Diagnostic value of FNAB varies according to the physicians who performed and evaluated the biopsy. CNB is a more invasive procedure and has complications such as hematoma, pain or discomfort. CNB is more accurate and reliable than FNAB but the definitive diagnostic method is excisional biopsy.

There are various management methods of juvenile fibroadenoma ranging from observation to surgical management. Conservative approach is usually favored in small fibroadenomas because they are not precursor for breast cancer, and about 10% to 40% of fibroadenomas resolve spontaneously [8]. There are various surgical or non-surgical treatment methods such as simple excision, cryoablation, vacuum-assisted excisional biopsy available to remove fibroadenomas [17]. Choosing the right technique is very important, especially in terms of aesthetic. Cryoablation has negative effects on pathological evaluation and may cause suspicious residual calcifications on mammograms. It also has size limitations to perform cryoablation. It can be applied to lesions larger than 4.2 cm in diameter [18]. Fibroadenomas which are less than 3 cm are removed by ultrasound-guided vacuum-assisted excisional biopsy [19]. Breast conservation is usually feasible for fibroadenomas. Mastectomy is an aggressive method and, if it is necessary, then nipple conserving methods are recommended. Reconstructive techniques generally come into prominence in multiple fibroadenomas, giant fibroadenomas or recurrent fibroadenomas. Mastopexy, reduction mammoplasty or augmentation are supplementary techniques which should also be evaluated individually. In our case, we performed Batwing mastopexy that is also called an inverted V or omega plasty because of the incision shape. We use a semi-circular line at the upper margin of the nipple areola complex (NAC) and another semi-circular parallel line above with two angled incisions to connect these two lines in a wing-like fashion to remove the lesion in this technique. The defect is closed by pulling up the inferior breast tissue. It may be used for lesions in the upper or central breast but it should be irrelevant with NAC [20]. During surgical excision, it is necessary to pay attention to the integrity of the fibroadenoma capsule. Disrupting the capsule of the fibroadenoma in surgical treatment increases the risk of recurrence and makes it difficult to define the borders in the case of malignancy or phyllodes tumor. The recurrence rate is about 33% at the 5-year follow-up after excision [21]. The surgical excision was performed with a safety margin of approximately 1 cm. During the 1-year follow-up, she was monitored with ultrasound scanning; no recurrence or deformity of the breast was reported and the patient is satisfied with the aesthetic result.

In conclusion, symmetry is an important feature in bilateral organs such as breasts. In the giant fibroadenoma with a significant breast asymmetry, excision with the Batwing mastopexy technique has succeeded in achieving symmetry with the opposite breast in these patients. The Batwing mastopexy technique provides easy access for tumor excision and also adjusts the position and the size of the NAC to maintain normal breast and symmetry.

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