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Research Article

# Effectiveness and safety of peroral endoscopic myotomy in patients with achalasia

## *Akalazya Hastalarında Peroral Endoskopik Miyotominin Etkinliği ve Güvenliği*

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## Abstract

**Aim:** This study aimed to present the outcomes in terms of efficacy and complications of the POEM procedure in adult patients diagnosed with achalasia and to investigate the potential effects of traditional risk factors such as age, gender, and obesity on these outcomes.

**Material and Methods:** A total of 51 patients who underwent a POEM procedure were retrospectively evaluated from January 2021 to July 2023. The main outcome measured was the rate of clinical success, determined by achieving an Eckardt score of 3 or lower two months after the procedure. Secondary outcomes involved any adverse events, ICU admissions, and the presence of reflux symptoms at the two-month post-procedure mark.

**Results:** The mean age of the patients was 49.3 ± 13.3 years, and the duration of symptoms ranged between 6 months and 10 years. Preoperative median Eckardt scores were 9, ranging between 5 and 12. At the 2nd month post-procedure, 96.1% of patients had an Eckardt score of 3 or lower, with a median reduction of 8 points (IQR = 6 – 8, $p < 0.001$). Among the patients, 13.7% encountered adverse events, comprising 3.9% with pneumomediastinum, 1.9% with mediastinitis, and 1.9% with intra-tunnel bleeding. At the 2nd month post-procedure, 17.6% of patients exhibited reflux esophagitis. No mortality was observed in any of the patients.

**Conclusion:** POEM is an effective, safe, and minimally invasive treatment for achalasia that represents a promising therapeutic option, offering symptomatic relief, improved quality of life, and boasting a high clinical success rate. Although a small percentage of patients experienced adverse events, these were manageable and did not result in mortality.

**Keywords:** Achalasia, endoscopy, per-oral endoscopic myotomy, myotom

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## ÖZ

**Amaç:** Bu çalışma, akalazya tanısı almış yetişkin hastalarda POEM prosedürünün etkinlik ve komplikasyonlar açısından sonuçlarını sunmayı ve yaş, cinsiyet ve obezite gibi geleneksel risk faktörlerinin bu sonuçlar üzerindeki potansiyel etkilerini araştırmayı amaçlamaktadır.

**Gereç ve Yöntemler:** Ocak 2021'den Temmuz 2023'e kadar POEM prosedürü geçiren toplam 51 hasta geriye dönük olarak değerlendirildi. Ana sonlanım noktası, prosedürden iki ay sonra Eckardt skorunun 3 veya daha düşük olmasıyla belirlenen klinik başarı oranı olarak değerlendirildi. İkincil sonlanım noktaları, herhangi bir advers olay, yoğun bakım ünitesine kabul ve prosedür sonrası iki ayda reflü semptomlarının varlığı olarak değerlendirildi

**Bulgular:** Hastaların ortalama yaşı 49,3 ± 13,3 yıl idi ve semptomların süresi 6 ay ile 10 yıl arasında değişiyordu. Ameliyat öncesi medyan Eckardt skorları 9 idi ve 5 ile 12 arasında değişiyordu. Prosedür sonrası 2. ayda hastaların %96,1'i 3 veya daha düşük bir Eckardt skoruna sahipti, medyan 8 puanlık bir azalma ile (IQR = 6 – 8, $p < 0.001$). Hastaların %13,7'si advers olaylarla karşılaştı, bunların %3,9'u pnömomediastinum, %1,9'u mediastinit ve %1,9'u tünel içi kanama içeriyordu. Prosedür sonrası 2. ayda hastaların %17,6'sı reflü özofajiti sergiledi. Hiçbir hastada mortalite gözlenmedi.

**Sonuçlar:** POEM, akalazya için etkili, güvenli ve minimal invaziv bir tedavi olup, semptomatik rahatlama, yaşam kalitesinde iyileşme sunan ve yüksek klinik başarı oranı ile umut verici bir terapötik seçenek olarak öne çıkmaktadır. Düşük oranda gözlenen advers olaylar yönetilebilir düzeydeydi ve mortaliteye neden olmadı.


**Anahtar Kelimeler:** Akalazya, endoskopi, peroral endoskopik miyotomi, miyotomi

## Introduction

Achalasia is a primary esophageal motility disorder characterized by the failure of the lower esophageal sphincter (LES) to relax and the loss of esophageal peristalsis. The disorder causes complaints like difficulty swallowing both solids and liquids, regurgitation, loss of weight, and chest pain [1]. Achalasia occurs at an incidence rate of between 0.3 and 1.63 cases annually per 100,000 adults, with a prevalence of 10 cases per 100,000 people each year [2, 3]. The existing treatment choices, including botulinum toxin injections, pneumatic dilation, Heller laparoscopic myotomy (LHM), and peroral endoscopic myotomy (POEM), are aim to ease symptoms and decreasing pressure in the lower esophagus. Among these approaches, especially POEM, has garnered considerable interest in recent years [4].

Introduced in 2008 as a minimally invasive procedure for treating achalasia, the use of POEM has rapidly spread worldwide. POEM offers efficacy comparable to surgical myotomy, leading to improvements in symptoms, esophageal emptying, and quality of life [5]. Recent meta-analysis studies have reported that POEM demonstrates high success rates and low complication risks in the treatment of achalasia [6, 7]. However, these studies also indicate that POEM can lead to complications such as pneumothorax or pneumoperitoneum, and they highlight the lack of sufficient data and inconsistent reporting on reflux esophagitis [6, 7]. Moreover, the success of POEM and its complication risks may be associated with traditional risk factors such as age, gender, or obesity [8]. On the other hand, there is still a lack of sufficient data on the long-term outcomes of POEM [9, 10].

This study aimed to present the outcomes in terms of efficacy and complications of the POEM procedure in adult patients diagnosed with achalasia and to investigate the potential effects of traditional risk factors such as age, gender, and obesity on these outcomes.

## Material and Methods

This retrospective study was conducted between January 2021 to July 2023 on adult achalasia patients at the Gastroenterology Clinic of XXXX Training and Research Hospital. The study was approved by the XXX Hospital's Ethics Committee Committee (Date: 10.08.2023, Decision No: 292) and was carried out in accordance with the relevant ethical guidelines and the Helsinki Declaration (2013 Brazil revision). Written informed consent was obtained from all participants.

This study involved 51 adult patients diagnosed with achalasia using high-resolution manometry (HRM) and treated with POEM. Patients with typical symptoms of achalasia such as dysphagia, regurgitation,

chest pain, and weight loss underwent endoscopic and radiological examinations. Following these examinations, HRM was applied, and the diagnosis and type of achalasia were determined according to the American College of Gastroenterology (ACG) guidelines [1]. The patients' demographic characteristics were recorded at the time of presentation. The patients were divided into three groups according to their ages: young adults (25-44 years), middle-aged adults (45-64 years), and old adults (≥65 years). The Eckardt score was calculated based on patient complaints [11]. Additionally, the pre-procedure weights, body mass indexes, and HRM findings of all patients were documented. HRM findings were classified as normal (Integrated Relaxation Pressure - IRP value <15 mmHg), suboptimal (due to being conducted with a water system HRM, issues related to the catheter, such as channel occlusion, as well as patient-related reasons for intolerance or lack of cooperation), and high (IRP ≥15 mmHg).

All patients were initiated on a liquid diet for 2 to 3 days before the procedure. In the 24 hours leading up to the procedure, they followed a nil per os (NPO) regimen and started on prophylactic antibiotics. The procedures were conducted with patients in a supine position and under general anesthesia. Initially, esophagogastroduodenoscopy (EGD) (Fujinon EG 590, Japan) was performed to pinpoint crucial anatomical markers. Carbon dioxide gas was used during the procedures. The gastroesophageal junction, known as the Z-line, was located first. Following this, a blend of normal saline and methylene blue was administered into the submucosal layer, 10 cm above this reference point. After the initial steps, a 2-cm longitudinal incision was created on the mucosal surface utilizing the Olympus triangle knife. The ESG 300 (Olympus, Japan) electrocautery device was used in all procedures. Subsequently, the same instrument's dissection and coagulation capabilities were employed to form a submucosal tunnel, which was extended down to 2 cm beneath the cardia, setting the stage for the myotomy phase. Pulse cut slow (effect 2/40 watt) mode and spray COA (effect 2/40 watt) mode was applied during submucosal tunneling. The posterior myotomy was executed using the ENDO CUT® Q setting, with Effect 3, a Cutting Duration of 2, and an Interval of 4. The incisions commenced 1 to 2 cm beneath the mucosal entry point and were extended towards the cardia, contingent upon the achalasia subtype. The extent of the myotomy varied, spanning 7 to 10 cm for Types I and II achalasia, and was adjusted according to manometric findings for Type III, generally reaching up to 12 cm. Prior to the procedure, the esophagus was cleansed with 20 mL of gentamycin. Subsequently, between five and seven hemostatic clips were applied to seal the mucosal incision. The procedural steps are depicted in Figure 1.

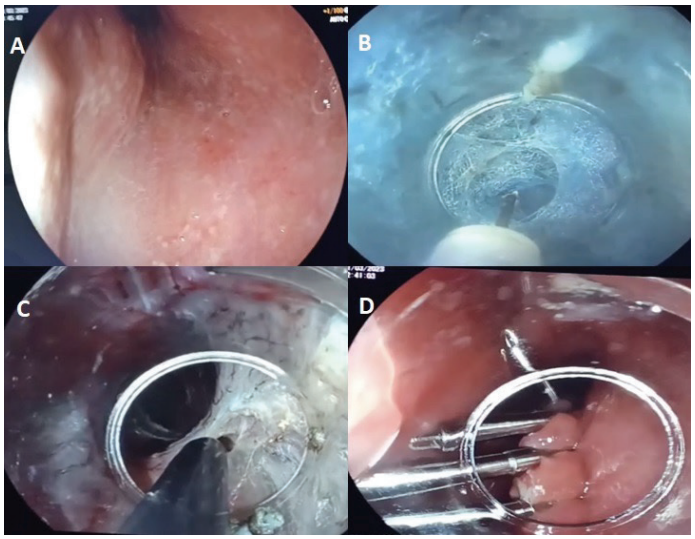


**Figure 1.** Steps of peroral endoscopic myotomy. **(A)** Esophageal mucosa before the intervention, **(B)** submucosal tunneling with the coagulation knife, **(C)** myotomy using the Olympus triangle knife and **(D)** mucosal closure with hemostatic clips

Post-procedure, all patients were extubated in the Endoscopy unit, maintained on NPO (nothing by mouth), initiated on analgesics and antiemetics, and continued on antibiotic treatment. All patients were administered triflow after 4-6 hours, and those who developed atelectasis achieved full recovery. In patients who developed pneumoperitoneum, decompression was achieved through underwater drainage, and this procedure was effective in all cases. In the initial 24-hour period post-procedure, the patient was monitored with no oral intake and followed by an endoscopic examination to assess the clips. Subsequently, the patient was allowed to consume clear liquids. Patients' oral intake was regulated for five days, and if no complications arose, they were discharged from the ward on the fifth day. Post-discharge, patients underwent endoscopic and HRM evaluations at the 1st and 3rd months. Concurrently, Eckardt scores and weight changes were also monitored.

## Statistical analysis

All analyses were conducted using IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA) and Medcalc 11.4.2 (MedCalc Software, Mariakerke, Belgium) software. The normal distribution of numerical variables was assessed using the Kolmogorov-Smirnov test. Data exhibiting a normal distribution were presented as mean±standard deviation, and

comparisons between groups were made using the Student's T-test. Non-normally distributed data were displayed as median (interquartile range (IQR): 25-75 percentiles) and comparisons between groups were conducted using the Mann-Whitney U test. Changes in clinical findings at the 3rd month post-operation were evaluated using the paired T-test for normally distributed data, the Wilcoxon test for non-normally distributed data, and the Marginal Homogeneity Test for categorical data. Value of $P < 0.05$ were considered statistically significant.

## Results

The ages of the patients ranged between 18 and 80 years (mean 49.3 ± 13.3 years), and the duration of symptoms ranged between 6 months and 10 years (median 3 years). The ratio of patients experiencing significant dysphagia symptoms to both solids and liquids was 86.3%. Nine patients (17.6%) had a history of balloon dilation, and two patients (4%) had a history of medical treatment. Of the patients, 5 (9.8%) had classic Type I achalasia, 44 (86.3%) had Type II achalasia, and 2 (3.9%) had Type III achalasia. Demographic and clinical characteristics of patients are shown in Tables 1.

**Table 1.** Demographic and clinical characteristics of patients.

| Variables | Results n = 51 |
|---|---|
| Age, years | 49.3 ± 13.3 |
| Gender, n (%) | |
| Female | 23 (45.1) |
| Male | 28 (54.9) |
| Height, cm | 168.4 ± 11.3 |
| Weight, kg | 70.4 ± 16.4 |
| BMI, kg/m2 | 24.6 ± 4.4 |
| Obesity, n (%) | 11 (21.6) |
| Duration of symptoms, years | 3 (1.5-5.0) |
| Dysphagia, n (%) | |
| Solid | 7 (13,7) |
| Solid & Liquid | 44 (86.3) |
| Time of diagnosis, year | 0.3 (0.2-1.0) |
| Type of achalasia, n (%) | |
| Type 1 | 5 (9.8) |
| Type 2 | 44 (86.3) |
| Type 3 | 2 (3.9) |
| Previous treatment, n (%) | |
| No | 40 (78.4) |
| Balloon dilatation | 9 (17.6) |
| Medical treatment | 2 (4.0) |

The data are expressed as the mean ± SD or median (IQR) or number (%). BMI, body mass index.

The POEM procedure was successfully carried out in all of the patients. The preoperative Eckardt scores of the patients varied, ranging from 5 to 12 points (median = 9, IQR = 7 –11). At the 2nd month post-procedure, 96.1% of patients had an Eckardt score of 3 or lower, ranged between 0 and 4 points (median = 0, IQR = 0 – 1) (Figure 2). The reduction in scores post-treatment varied from 1 to 12 points, with the median reduction being 8 points (IQR = 6 – 8, $p < 0.001$). Additionally, there was a significant increase in the mean weight of the patients at the two-month post-operation (Table 2).

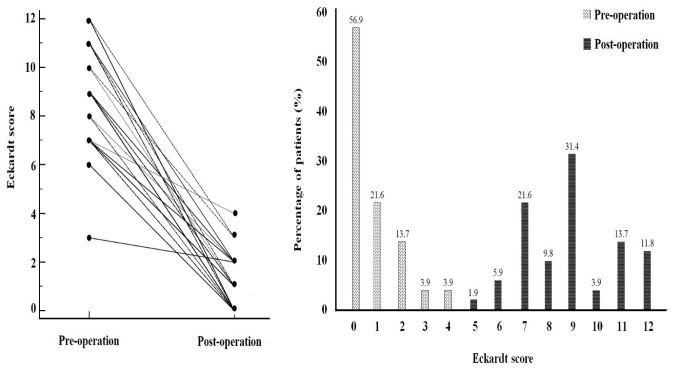


**Figure 2.** Post-operative Eckardt score changes and distributions

**Table 2.** Changes in the clinical findings of the patients during their follow-up.

| Variables | Baseline n = 51 | 3 months Post-operation n = 51 | p |
|---|---|---|---|
| Eckardt score | 9 (7-11) | 0 (0-1) | <0.001* |
| HRM / IRP, n (%) | | | |
| Normal | - | 49 (95.1) | <0.001* |
| Suboptimal | - | 2 (3.9) | |
| High | 51 (100.0) | - | |
| Weight, kg | 70.4 ± 16.4 | 71.1 ± 14.6 | <0.001* |

The data are expressed as the mean ± SD or median (IQR) or number (%). BMI, body mass index; HRM, high-resolution manometry; IRP,

In terms of adverse events, no complications were detected in 44 patients (86.3%). Among the seven patients with complications, atelectasis was detected in three, pneumomediastinum in two, mediastinitis in one, and intra-tunnel bleeding in another. The patient who experienced intra-tunnel bleeding was monitored in the ICU (Table 3). Following successful management of the bleeding, the patient was subsequently discharged. No mortality was observed in any of the patients. The median operation time was 40 minutes (range: 25-60 minutes), and the median hospital stay was 8 days (range: 5-12 days). During their follow-up, reflux esophagitis was identified in 9 patients (17.6%) (Table 3).

**Table 3.** Adverse events after the operation and during the two-month follow-up period.

| Variables | Results n = 51 |
|---|---|
| Complication, n (%) | 7 (13.7) |
| Atelectasia | 3 (5.9) |
| Pneumomediastinum | 2 (3.9) |
| Mediastinitis | 1 (1.9) |
| Intra-tunnel bleeding | 1 (1.9) |
| Hospitalized in ICU, n (%) | 1 (1.9) |
| Two-month follow-up | |
| Reflux esophagitis, n (%) | 9 (17.6) |

The data are expressed as number (%). ICU, intensive care unit.

In those who developed complications compared to those who did not, the rate of young adults was higher (54.2% vs. 20.5%, $p < 0.05$), while the rate among middle-aged adults was lower (14.3% vs. 54.5%, $p < 0.05$). Other demographic and clinical features did not show a correlation with the development of complications (Table 4).

**Table 4.** Findings related to complications.

| Variables | Complication | | p |
|---|---|---|---|
| | No n = 44 | Yes n = 7 | |
| Age, years | 50.5 ± 12.2 | 41.3 ± 17.8 | 0.086 |
| Young adult | 9 (20.5) | 4 (54.1) | 0.047* |
| Middle-aged adults | 24 (54.5) | 1 (14.3) | |
| Old adults | 11 (25.0) | 2 (28.6) | |
| Gender, n (%) | | | |
| Female | 21 (47.7) | 2 (28.6) | 0.591 |
| Male | 23 (52.3) | 5 (71.4) | |
| Height, cm | 167.8 ± 10.7 | 171.9 ± 15.1 | 0.389 |
| Weight, kg | 70.9 ± 17.2 | 67.0 ± 10.4 | 0.562 |
| BMI, kg/m2 | 25.0 ± 4.6 | 22.7 ± 2.7 | 0.216 |
| Obesity, n (%) | 10 (22.7) | 1 (14.3) | 0.992 |
| Duration of symptoms, years | 3 (1.5-5.0) | 3 (1.5-4.0) | 0.862 |
| Dysphagia, n (%) | | | |
| Solid | 8 (18.2) | - | 0.503 |
| Solid & Liquid | 36 (81.8) | 7 (100.0) | |
| Time of diagnosis, year | 0.3 (0.2-1.0) | 0.2 (0.1-0.4) | 0.102 |
| Type of achalasia, n (%) | | | |
| Type 1 | 5 (11.4) | - | 0.364 |
| Type 2 | 38 (86.4) | 6 (85.7) | |
| Type 3 | 1 (2.3) | 1 (14.3) | |
| Previous treatment, n (%) | | | |
| No | 34 (77.3) | 6 (85.7) | 0.225 |
| Balloon dilatation | 9 (20.5) | - | |
| Medical treatment | 1 (2.3) | 1 (14.3) | |
| Eckardt score | 9 (7-10) | 9 (8-11) | 0.445 |

The data are expressed as the mean ± SD or median (IQR) or number (%). * P <0.05 shows statistical significance. BMI, body mass index.

The rate of solid and liquid dysphagia was higher in those who developed reflux esophagitis compared to those who did not (54.2% vs. 20.5%, $p < 0.05$). Other demographic and clinical features did not show a correlation with the development of reflux esophagitis (Table 5).

**Table 5.** Findings related to reflux esophagitis.

| Variables | Reflux esophagitis | | p |
|---|---|---|---|
| | No n = 44 | Yes n = 7 | |
| Age, years | 50.0 ± 13.0 | 46.0 ± 12.8 | 0.420 |
| Young adult | 11 (26.2) | 2 (22.2) | 0.521 |
| Middle-aged adults | 19 (45.2) | 6 (66.7) | |
| Old adults | 12 (28.6) | 1 (11.1) | |
| Gender, n (%) | | | |
| Female | 21 (50.0) | 2 (22.2) | 0.250 |
| Male | 21 (50.0) | 7 (77.8) | |
| Height, cm | 168.0 ± 11.0 | 170.7 ± 13.4 | 0.512 |
| Weight, kg | 70.0 ± 17.0 | 71.7 ± 16.9 | 0.801 |
| BMI, kg/m2 | 24.7 ± 4.7 | 24.3 ± 3.0 | 0.802 |
| Obesity, n (%) | 9 (21.4) | 2 (22.2) | 0.999 |
| Duration of symptoms, years | 3 (1.5-4) | 3 (1-5) | 0.971 |
| Dysphagia, n (%) | | | |
| Solid | 4 (9.5) | 4 (44.4) | 0.035* |
| Solid & Liquid | 38 (90.5) | 5 (55.6) | |
| Time of diagnosis, year | 0.3 (0.2-1.0) | 0.3 (0.2-0.5) | 0.761 |
| Type of achalasia, n (%) | | | |
| Type 1 | 4 (9.5) | 1 (11.1) | 0.478 |
| Type 2 | 37 (88.1) | 7 (77.8) | |
| Type 3 | 1 (2.4) | 1 (11.1) | |
| Previous treatment, n (%) | | | |
| No | 35 (83.3) | 5 (55.6) | 0.029* |
| Balloon dilatation | 7 (16.7) | 2 (22.2) | |
| Medical treatment | - | 2 (22.2) | |
| Eckardt score | 9 (7-10) | 9 (7-11) | 0.894 |
| Complication, n (%) | 6 (14.3) | 1 (11.1) | 0.999 |

The data are expressed as the mean ± SD or median (IQR) or number (%). * P <0.05 shows statistical significance. BMI, body mass index.

## Discussion

POEM was first introduced by Ortega et al. in 1980, marking a significant milestone in the treatment of achalasia. This pioneering approach showed promising results; the patients in this cohort experienced symptom improvement that was on par with the outcomes of Heller myotomy, a more established surgical intervention at the time [12]. However, it wasn't until 2010 that Inoue et al. significantly refined this procedure and presented it as a groundbreaking alternative for treating achalasia [13]. Prior to POEM's introduction, various treatment strategies were commonly employed. Some of these were

conservative, involving medications like calcium channel antagonists and nitrates to lower esophageal pressure. Others targeted the lower esophageal sphincter (LES) more directly, such as endoscopic pneumatic dilatation, botulinum toxin injection, or Heller's myotomy. Each of these traditional methods had its limitations [14]. Among the frequently used treatments, pneumatic dilation was linked with the recurrence of symptoms and an increased risk of gastroesophageal reflux disease (GERD) following the procedure [15, 16]. Botulinum toxin injections, another minimally invasive approach feasible under endoscopic guidance, only offered short-term relief, necessitating repeat procedures which could culminate in substantial treatment costs [17]. Surgical myotomy, though highly effective, came with its own set of drawbacks. It was invasive, required hospitalization, often entailed an additional fundoplication procedure to reduce the likelihood of postoperative GERD, and carried a risk of intraoperative esophageal perforation [18].

The minimally invasive approach, brief duration of hospitalization, and enduring therapeutic outcomes have made POEM a widely accepted alternative therapy for achalasia [6, 7]. In our study, POEM achieved a technical success rate of 100% and a clinical success rate of 96%, which corresponds with the rates mentioned in previous studies [6, 7, 19-22]. Despite the short follow-up period in our study, studies with follow-up periods between 1-5 years have demonstrated that POEM maintains a consistent clinical success rate within the range of 82-90% [23-26]. Moreover, this success rate remained unchanged even in patients who had previously received treatment. This consistency with previous studies reporting the safety and efficacy of POEM in complex achalasia cases [23, 24, 27, 28]. The rate of complication in our study (13.7%) was within the range reported in the literature (6% to 17%) [7, 29, 30]. Atelectasis was observed in 6% of the cases and was the most common complication. Studies involving both pediatric and adult patients has indicated that the incidence of atelectasis ranges from 7% to 21% [30-33]. Studies indicate that this adverse event, as well as pneumomediastinum, could be associated with gas-related complications [33, 34]. On the other hand, the rate of complications such as infection or bleeding in POEM procedures is notably low, typically ranging between 0.3% to 2.7% [35-37]. Besides, the prevalence of GERD, a significant long-term adverse event of POEM, has been reported in various meta-analysis studies to range between 8.5% and 19% [38-40]. In this study, the frequency of Reflux esophagitis was 17.6% during the two-month follow-up of the patients. While the adverse events profile of this study indicates that POEM is generally safe, it also underscores that both short- and long-term outcomes of POEM can be closely associated with the patient's profile.

Some studies have reported that male gender, either young or advanced age, manometric subtype 3, and duration of symptoms might be potential risk factors in the prognosis of achalasia patients treated with POEM [9, 30, 41-43]. However, there are studies that report contrary findings [30, 42, 44]. In the current study, neither the duration of symptoms nor previous treatments were associated with the development of complications. However, younger adult patients exhibited a higher rate of complications. Moreover, the majority of patients who developed complications belonged to this age group. On the other hand, male patients and those with type 3 achalasia were also found to be more prone to complications. In addition to patients who had received previous treatments, these patients were also susceptible to reflux esophagitis [45, 46]. Therefore, despite high success rates of POEM in various patient profiles, there may be a need for criteria specific to patient selection concerning both short- and long-term complications.

The current study had several notable limitations. The study primarily had a single-center, retrospective design and involved a comparatively small cohort of subjects. Secondly, the study was characterized by a short follow-up period. Lastly, perioperative findings were not included in the study. These factors could potentially introduce bias in assessing both the success and the short and long-term outcomes of POEM, affecting the identification of potential risk factors.

## Conclusion

POEM is an effective, safe, and minimally invasive treatment for achalasia that represents a promising therapeutic option, offering symptomatic relief, improved quality of life, and boasting a high clinical success rate. Although a small percentage of patients experienced adverse events, these were manageable and did not result in mortality. The occurrence of reflux esophagitis in a subset of patients highlights the need for ongoing monitoring and management of potential postoperative complications.

Conflict of Interest/ Funding: Funding: The study received no financial support from any individual or organization, and the authors declare no conflict of interest.

## Ethics Approval

The study was performed in accordance with the Declaration of Helsinki, and was approved by the Umraniye Training and Research Hospital Clinical Research Ethics Committee (Date: 10.08.2023, Decision No: 292).

## Informed Consent

The need for informed consent was waived under the approval of the Local Ethics Committee due to the retrospective design.

Availability of Data and Material: The data that support the findings of this study are available on request from the corresponding author.

## Authors' contribution

Concept – N.M.B. and K.O., Design- N.M.B., Z.C., and K.O., Supervision - Z.C., Data collection and/or processing - N.M.B., Z.C., M.A.S., O.O., and K.O., Analysis and/or interpretation - N.M.B., Z.C., M.A.S., O.O., and K.O., Writing – N.M.B., Critical review- Z.C., M.A.S., O.O., and K.O. All authors read and approved the final version of the manuscript.

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