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A combined treatment strategy of Legg-Calve-Perthes disease with BEST quartet

Legg-Calve-Perthes hastalığının BEST dörtlüsü ile bir kombine tedavi stratejisi

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Abstract

Purpose: The main pathology of Legg-Calvé-Perthes Disease (LCPD) is the disruption of blood flow of the femoral head resulting ischemic necrosis which leads hip joint incongruency. The most frequent methods in the treatment are the methods improving the containment of the femoral head. There are limited studies in the literature addressing the treatment of necrosis using methods such as epiphyseal drilling or distraction osteogenesis. Our study aimed to investigate the mid-long-term outcomes of LCPD patients treated with the BEST method, which aims to improve congruency and containment simultaneously.

Materials and methods: LCPD patients (23 male, 1 female) who were treated with (B)leeding the epiphysis by drilling, (E)vacuation of the joint synovitis, contained with (S)alter's Osteotomy, and distracted with skin (T)raction investigated retrospectively. Only patients who reached skeletal maturity at latest follow-up were included in the study. Patients' final radiographs were classified according to the Stulberg classification, Mose classification, and Tönnis osteoarthritis classification.

Results: The mean follow-up of the patients was 10.44±1.35 (8.5-13) years, and the mean age at the last follow-up was 17.71±1.73 (15.25-20.83) years. According to the Stulberg classification, 11 (45.8%) of the patients had a Class-I hip; 6 (25%) a Class-II hips; 3 (12.5%) a class-III hips, 4 (16.7%) a class-IV hips. According to the Mose classification, 12 (50%) of the patients had good results; 5 (20.8%) had fair results; 7 (29.1%) had poor results.

Conclusion: BEST treatment method for LCPD is a combined procedure which addresses to all pathologies of disease at the same time. This combined treatment protocol may be preferred for severely affected LCPD cases who with subluxation especially in higher lateral pillar class that are prone to nonspherical incongruency.

Keywords: Legg-Calve-Perthes disease, Salter's osteotomy, femoral head osteonecrosis.

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Öz

Amaç: Legg-Calvé-Perthes hastalığının (LCPH) temel patolojisi, femur başındaki kan akışının bozulması sonucunda kalça ekleminde uyumsuzluğa yol açan iskemik doku nekrozudur. Tedavide sık uygulanan yöntemler femur başının kapsamasını artıran yöntemlerdir. Epifizin delinmesi veya distraksiyon osteogenezi gibi nekroz tedavisine değinen nadir yayın vardır. Çalışmamızın amacı, uyum ve kapsamayı aynı anda geliştirmeyi amaçlayan BEST yöntemiyle tedavi ettiğimiz LCPH hastalarının orta-uzun dönem sonuçlarını araştırmaktı.

Gereç ve yöntem: LCPH tanısı ile 24 (23 erkek, 1 kadın) hastaya uygulanan epifizi delme yoluyla kanlandırma (B), eklem ponksiyonu ile fazla eklem sıvısının boşaltılması (E), Salter Osteotomisi (S) ile femur başı kapsamasının artırılması ve cilt (T)raksiyonu ile eklemdeki basıncın azaltılması yöntemlerinin birlikte kullanıldığı kombine yöntem araştırıldı. Çalışmaya sadece iskelet olgunluğuna ulaşan hastalar dahil edildi. Hastaların son radyografileri Stulberg sınıflaması, Mose sınıflaması ve Tönnis osteoartrit sınıflamasına göre sınıflandırıldı.

Bulgular: Hastaların ortalama takip süresi 10,44±1,35 (8,5-13) yıl, son takip yaş ortalaması ise 17,71±1,73 (15,25-20,83) yıldı. Stulberg sınıflamasına göre hastaların 11'inde (%45,8) sınıf I kalça, 6 hastada (%25) sınıf II kalça, 3 hastada (%12,5) sınıf III kalça, 4 hastada (%16,7) sınıf IV kalça görüldü. Mose sınıflamasına göre hastaların 12'sinde (%50) iyi sonuç, 5'inde (%20,8) orta sonuç, 7'sinde (%29,1) kötü sonuç elde edildi.

Sonuç: LCPH için BEST tedavi yöntemi, hastalığın tüm patolojilerine aynı anda hitap eden kombine bir prosedürdür. Bu kombine tedavi protokolü, özellikle asferik uyumsuzluğa eğilimli, yüksek lateral pillar sınıfında, subluksasyonlu ve ciddi şekilde etkilenmiş LCPH vakalarında tercih edilebilir.

Anahtar kelimeler: Legg-Calve-Perthes hastalığı, Salter osteotomisi, femur başı osteonekrozu.

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Introduction

The main pathological event of Legg-Calvé-Perthes Disease (LCPD) is the disruption of 3 blood flow of the femoral head resulting in ischemic tissue necrosis [1]. After a century from the first description, a widely accepted treatment algorithm could not be established yet. Improving the containment (surgically or conservatively) is a frequently used method to reduce the local loads on the femoral head to prevent deformity and obtain a spherical and congruent joint using the molding effect of the acetabulum during the healing period [2-14].

Recently, publications have reported that drilling can be used in LCPD, which is also used in adult osteonecrosis of the femoral head [15-18]. However, the drawback of drilling is that it may cause physeal damage, resulting in premature physeal closure and consequent deterioration in growth. However, Park et al. [19] observed premature physeal closure in 21 (77.8%) of 27 unilateral LCPD patients treated conservatively and stated that premature physeal closure was associated with the Herring class of the disease (more in Herring B/C and 14 C), but not the Stulberg class.

It may be possible to increase the success rate by combining various methods in treating LCPD. Thus, drilling of the necrotic area (to reduce intraosseous pressure and enhance revascularization), evacuation of hip joint excess fluid (to reduce intra-articular pressure), Salter's osteotomy (to increase containment), and skin traction (to reduce intra-articular pressure and to obtain distraction osteogenesis) can be performed together. We abbreviate this combination as BEST. B is bleeding by drilling, E is the evacuation of the excessive joint fluid, S is Salter's osteotomy and T is skin traction. The present study aimed to investigate the mid-long-term outcomes of patients with LCPD that we treated with the BEST method retrospectively.

Material and methods

Our indications for BEST treatment in LCPD are; (1) symptoms started after the age of six (2) severe involvement of the epiphysis of the femoral head (Catterall 3-4 or lateral pillar B, B/C, C) (3) subluxation of the femoral head (regardless of the involvement of the femoral head). These patients got indications whenever they showed these criteria (first admission or at follow-ups).

BEST procedure

All patients were operated on and treated by a single physician (ET). After routine preparation, adductor tenotomy was performed with a mini-incision in all cases. The necrotic area in the femoral head was drilled by 1.5 mm smooth Kirschner wire from the lateral trochanteric region percutaneously under fluoroscopy (BLEEDING) (Figure 1). Drilling was administered twice or three times depending on the size of the necrosis from the same entry points but in different directions. An anterior iliofemoral approach was used for Salter's innominate osteotomy. Hip joint fluid evacuation was performed before osteotomy by a needle (EVACUATION) (Figure 1). The classical Salter's innominate osteotomy was administered using a Gigli saw (SALTER) (Figure 1). No rigid immobilization method was used, including a spica cast. Skin traction was applied to the extremity and 0.5 kg weight was suspended from the distal to ensure continuous traction on the joint (TRACTION) (Figure 1). Skin traction was discontinued for the 4th week (the first three days in the hospital, then at home). At the end of four weeks, skin traction was terminated and passive joint movements were started. Mobilization by partial weight-bearing with crutches and active ROM exercises was started at the end of the 8th week. Full weight-bearing was allowed at the end of the 12th week. After the 12th week, the patients were followed-up at 3-month intervals in the first year and then at 6-month intervals until the end of the healing period. After mobilization, parents were informed about activity restrictions and not being interested in heavy sports.

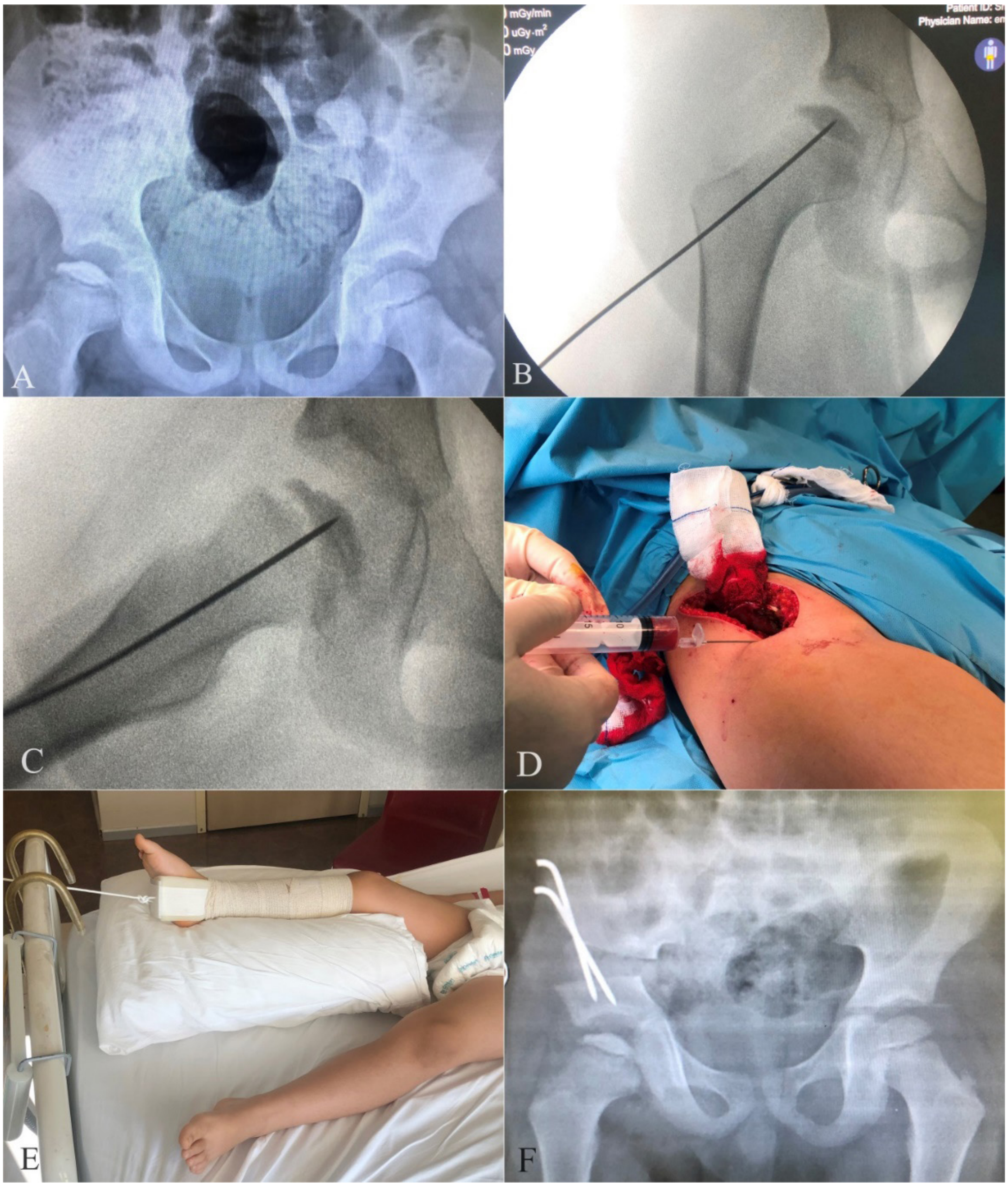

Figure 1. A seventy-three months of age boy with LCPD in his right hip (A). The patient who was decided to apply BEST treatment; The epiphysis of the femoral head was drilled with a 1.5mm Kirschener wire and its blood supply was increased (Bleeding) (B, C). Excess and harmful fluid in the hip joint is evacuated (Evacuation) (D). After the Salter innominate osteotomy was performed, the patient's right lower extremity was placed under skin traction (Traction) (E). Post-operative radiograph of the patient (Salter Innominate osteotomy) (F)

Note the increased the containment of femoral head by acetabulum after the Salter innominate osteotomy (This patient was not included in the study)

Study design

Ethics committee approval was obtained before this retrospective study. Patients diagnosed with LCPD in our hospital and treated with the BEST method were investigated. Twenty-five patients (twenty-four boys, one girl) who reached skeletal maturity were included in this study. Patients were invited to the hospital for final evaluation except one (who couldn't be reached). Twenty-four patients (twenty-three boys, one girl) participated in the final evaluation. Informed consent was obtained from the patients for the present study.

Clinical evaluation

Pre- and post-treatment examinations and surgical notes taken from hospital records were analyzed. In the final physical evaluation, range of motion and leg lengths were measured, and the Trendelenburg sign was noted for each patient. In addition, in their last follow-up, the patients were asked to fill a questionnaire for the Harris Hip Score (HHS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). The duration of surgery, the amount of bleeding and complications were obtained from the surgery notes.

Radiological evaluation

The radiographs of the patients before the treatment were all obtained and examined from the hospital records. Pelvic X-rays were taken in the AP and frog-leg positions of the patients in their final evaluations. The preoperative radiographs were classified according to the lateral pillar classification [10], the Catterall [2] classification, and the Waldenström [20] staging. The presence and number of risk signs (lateral calcification, lateral subluxation, gage sign, horizontal growth plate and diffuse metaphyseal reaction) indicated by Catterall [2] were investigated and Wiberg's Central Edge (CE) angle [21] was measured pretreatment radiographs. Final radiographs were classified according to the Stulberg et al. [22] classification and Tönnis osteoarthritis classification [23]. The sphericity of the femoral head was evaluated using the Mose [24] method. The result was considered good if the femoral head was spherical in the AP and lateral projections with no deviation of the concentric rings. A deviation of 2 mm or less was considered fair and a deviation greater than 2 mm was considered poor. CE angles were measured on the final radiographs again. In the final evaluation, the Risser [25] index was considered the criterion for patients to reach skeletal maturity.

To provide an accurate and reliable radiographic evaluation, two independent experienced orthopedic surgeons (one was in treatment and study, one was not) and a radiologist classified the radiographs according to the Stulberg classification, and the Herring classification independently. Both intra- and inter-observer reliability radiographic evaluations were performed three weeks apart.

Statistical analysis

Categorical variables were expressed as numbers and percentages, and continuous variables were summarized as median and IQR. The chi-square test was used to compare categorical variables between the groups. The normality of distribution for continuous variables was confirmed with the Shapiro-Wilk test. For comparison of continuous variables between two groups, Mann-Whitney U test was used. For non-normal distributed data, the Kruskal Wallis test was used to compare more than two groups. Bonferroni adjusted Mann-Whitney U test was used for multiple comparisons of groups. For comparison of preop-postop CE angle values, Wilcoxon signed-rank test was used. To evaluate the correlations between measurements, Spearman Rank Correlation Coefficient was used. To measure intra- and inter- observer reliability, Intraclass Correlation Coefficient (ICC) was used. All analyses were performed using IBM SPSS Statistics Version 20.0 statistical software package. The statistical level of significance for all tests was considered to be 0.05.

Results

The patients (23 males, 1 female) included in this study were with unilateral (6 right, 18 left) involvement and a mean age of 7.3±1.12 (6.16-9.91) years at the time of surgery. All the patients had limp and/or hip pain and limited hip joint movements during the examination before surgery. Pre-treatment radiographic findings of the hips are given in Table 1.

Table 1. Pre-treatment radiographic findings and measurements of hips

	n (%)
Waldenström Staging	
Initial (Necrosis)	6 (25%)
Fragmantation	18 (75%)
Catterall Classification	
3	7 (29%)
4	17 (71%)
Lateral Pillar Classification	
B	9 (37.5%)
B/C	0 (0%)
C	15 (62.5%)
Number of Risk Signs	
1	6 (25%)
2	9 (37.5%)
3	2 (8.3%)
4	6 (25%)
5	1 (4.2%)

The mean surgery time was 74.3±10.2 (50-90) minutes, and the mean amount of bleeding during surgery was 77.9±10.9 (60-100) ml. None of the patients experienced complications during the surgery and did not need a transfusion. There was no wound healing problem or infection detected in the follow-ups. Implant removal procedures were routinely performed 6-8 months after surgery.

The mean follow-up period of the patients was 10.44±1.35 (8.5-13) years, and the mean age at the last follow-up was 17.71±1.73 (15.25-20.83) years. In the last follow-up examination, an average of 5.2±2.13 (4-10) mm limb shortening was detected clinically in only seven (29.1%) patients and limited joint rotation in four (16.7%) patients. Trendelenburg sign was present in five (20.8%) patients. The mean HHS was 94.9±11.4 (54-100), and the mean WOMAC index was 5.3±13 (0-53). According to the HHS grading system, 20 patients (83.3%) had an excellent outcome; one patient (4.2%) had a good result; two patients (8.3%) had a fair result; and one patient (4.2%) had a poor result (Figure 2).

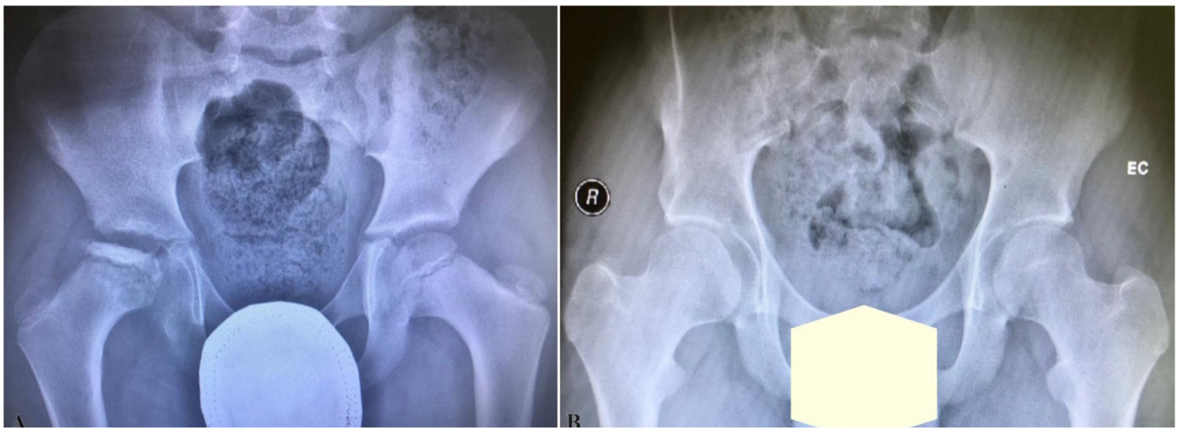

Figure 2. A 6.1 years old age boy had been sustained from hip pain for 4 months and was limping while walking

After the examination, it was determined that there was LCPD in the right hip (A). There was more than 50% depression in lateral pillar (lateral pillar C). The femoral head had three radiological risk factors; diffuse metaphyseal reaction, lateral calcification and lateral subluxation. Radiograph after 9.5 years after BEST treatment, showing good remodeling of the femoral head (B)

All radiological results are given in Table 2. Inter-observer variability revealed no significant difference in lateral pillar classification and Stulberg classification measurements between the observers (ICC >0.90). Likewise, intra-observer variability showed no significant differences between measurements taken by the same observers (ICC >0.90). No significant relationship was noted in the analysis between the age of surgery, follow-up duration, Waldenström stage [20], Catterall class and number of risk signs on Stulberg classification. However, the relation between lateral pillar classification and Stulberg classifications showed that Class B hips of lateral pillar classification ended in a better Stulberg class than Class C hips (Table 3). This relationship was also valid for the Mose [24] index (Table 4) (Figure 3). The CE angles of the patients at the last follow-up were higher than before the operation (p<0.001) (Figure 4).

Table 2. Final radiographic measurements and results

	n (%)
Stulberg Classification	
Class 1	11 (45.8%)
Class 2	6 (25%)
Class 3	3 (12.5%)
Class 4	4 (16.7%)
Class 5	0 (0%)
Mose Classification	
Good	12 (50%)
Fair	5 (20.8%)
Poor	7 (29.1%)
Presence of Osteoarthritis	
Yes	6 (25%)
Tönnis Grade 1	5 (20.8%)
Tönnis Grade 2	1 (4.2%)
No	18 (75%)

Table 3. Analysis of Stulberg classification

	Stulberg Class				***p statistics***
	1	**2**	**3**	**4**	
Age at surgery, years, median (IQR)	7 (1.45)	6.5 (2.09)	6.4 (1.1)	7.3 (2.19)	0.737 KW:1.265
Follow-up time, years, median (IQR)	10 (1)	10.5 (1.5)	10 (1.5)	10.7 (2.3)	0.573 KW:1.998
Waldenström stage					
Initial (necrosis)	2 (33.3%)	2 (33.3%)	2 (33.3%)	0 (0%)	0.198
Fragmantation	9 (50%)	4 (22.2%)	1 (5.5%)	4 (22.2%)	FE:4.004
Catterall class					
3	6 (85.8%)	1 (14.2%)	0 (0%)	0 (0%)	0.178
4	5 (29.4%)	5 (29.4%)	3 (17.6%)	4 (23.5%)	FE:4.449
Lateral pillar class					
B	8 (89%)	1 (11%)	0 (0%)	0 (0%)	**0.011**
C	3 (20%)	5 (33%)	3 (20%)	4 (27%)	FE:9.714
Risk sign number, median (IQR)	2 (1)	3 (3)	2 (0)	3 (2)	0.382 KW:2.527

KW: Kruskall Wallis Test, FE: Fisher-Exact Test

Table 4. Analysis of Mose classification

	Mose Class			***p* Statistics**
	Good	**Fair**	**Poor**	
Age at surgery, years, median (IQR)	7 (1.7)	7.5 (2)	6.8 (1.7)	0.958 KW:0.087
Follow-up duration, years, median (IQR)	10.1 (1.1)	10.6 (1.5)	10.5 (1.9)	0.558 KW:1.166
Waldenström stage				
Initial (necrosis)	2 (33.3%)	2 (33.3%)	2 (33.3%)	0.585 FE:1.357
Fragmantation	10 (55.5%)	3 (16.7%)	5 (27.8%)	
Catterall class				
3	6 (85.8%)	1 (14.2%)	0 (0%)	0.064 FE:5.249
4	6 (35.3%)	4 (23.6%)	7 (41.1%)	
Lateral pillar class				
B	8 (88.9%)	1 (11.1%)	0 (0%)	**0.010** FE:8.903
C	4 (26.7%)	4 (26.7%)	7 (46.6%)	
Risk sign number, median (IQR)	2 (2)	2 (3)	3 (2)	0.391 KW:1.876

KW: Kruskall Wallis Test, FE: Fisher-Exact Test

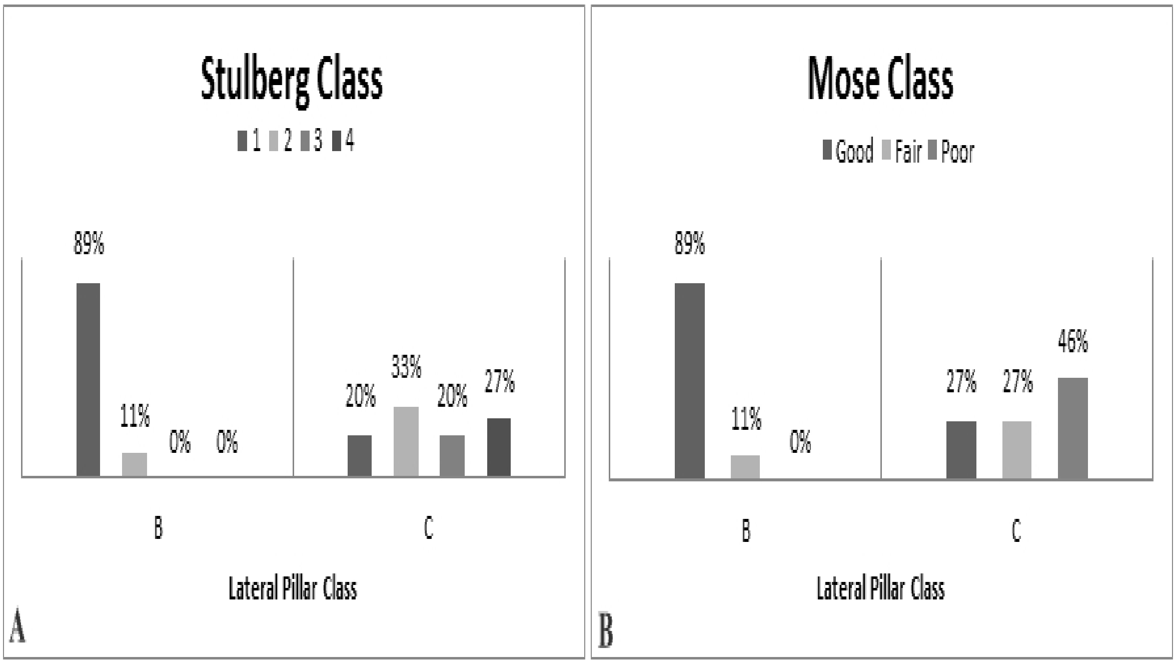

Figure 3. Lateral pillar classes according to Stulberg classes (A) and Mose classes (B)

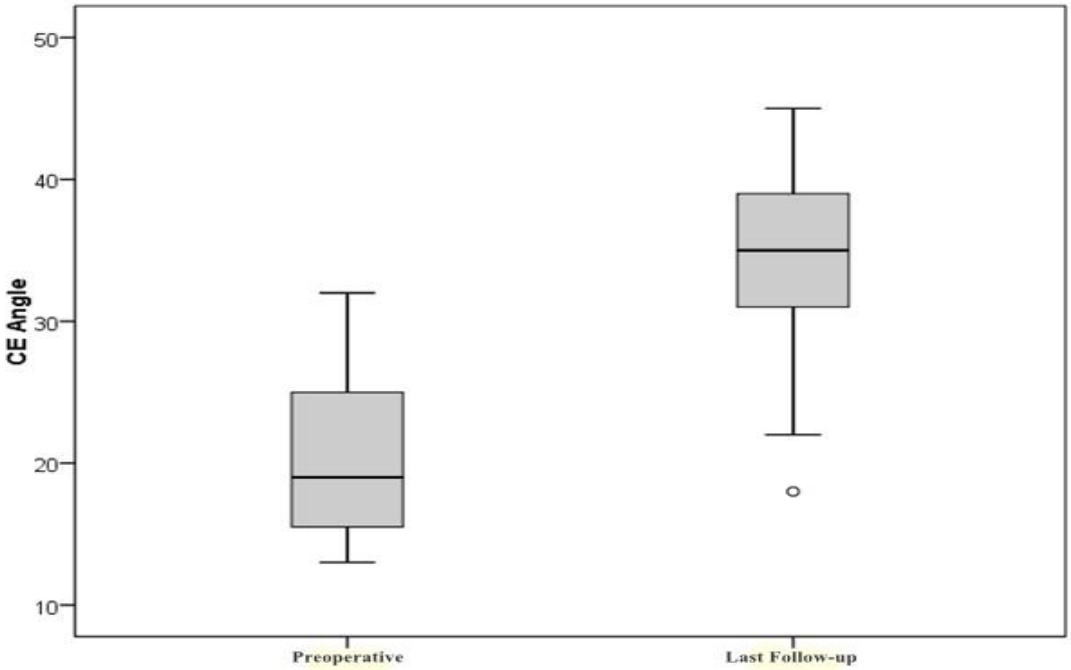

Figure 4. The CE angle showing centralization of the femoral head was higher at the last follow-up than before the operation

Preoperative CE angle Median: 19 (IQR:10), Last follow-up CE angle Median:35 (IQR:9), *p*<0.001

Discussions

Although more than a century has passed since the definition of the LCPD a common consensus has not been established on its treatment yet. The ultimate goal of treatment is to achieve a spherical and congruent hip joint. Joseph et al. [26] reported that spherical femoral head development could not be achieved in 76% of untreated LCP patients. The most accepted and practiced method is to improve the containment of the head by the acetabulum by the molding effect of the socket during the period when the head is susceptible to deformation. Many conservative and surgical methods can be applied to increase containment [2-14].

In treating of LCPD, brace and orthosis can be used as conservative methods to increase containment [27, 28]. Rich and Schoenecker [27] stated that 89% of lateral pillar B and 67% of lateral pillar C hips were spherical and congruent at maturity by orthosis. However, Wiig et al. [28], in their study comparing femoral varus osteotomy and orthosis treatment, reported that 43% spherical and congruent hips were obtained in patients older than six years of age who underwent surgery, while this rate was 20% in orthosis treatment. Terjesen et al. [29] stated that surgical treatment could be more successful in patients with Catterall 4, Lateral pillar C, more than six years of age, and less than 80% femoral head coverage, which they identified as risk factors.

Pelvic osteotomies could be performed to increase containment, including Salter innominate osteotomy, triple pelvic osteotomy, Chiari osteotomy, and shelf osteotomy [3, 4, 6, 7, 13, 30, 31]. Salter innominate osteotomy further increases the coverage of the anterolateral part of the femoral head, where the load is higher. Its advantages are that it medializes the acetabulum by 1-1.5 cm, reduces the load on the joints, increases blood supply to the femoral head, does not cause shortening, and easy remove implants [4]. In the literature reporting the results of Salter's Osteotomy in the treatment of LCPD, it is not possible to make a direct comparison because many factors, such as the timing of the treatment, severity of the disease, and age, cannot be standardized. In various small series published, the rate of obtaining excellent-good results with Salter osteotomy (Stulberg 1-2) varies from 46% to 74% [6-8, 11]. In a series of 35 patients with a mean follow-up of 9.4 years, where Kaneko et al. [12] reported 74% excellent and good results, 20% of the patients were in the lateral pillar C group. Volpon [8] reported 46% good-excellent results in their series of 28 cases. However, there are no data about the number of patients in the lateral pillar groups in their publications. In our series, of 24 patients, 11 (45.8%) patients were Stulberg Class-1, six (25%) were Stulberg Class-2, 3 (12.5%) were Stulberg Class-3, and 4 (16.7%) were Stulberg Class-4. Fifteen of these patients were in lateral pillar C class. All nine patients with lateral pillar class B were excellent or good (8 Stulbeg-1 class, 1 Stulberg-2 class).

Recently, studies in the treatment of LCPD have focused on shortening the course of the disease and accelerating regeneration. Oh et al. [31] stated that the prolongation of the initial and fragmentation phases affected the results negatively, and that especially shortening the fragmentation period would positively affect the results. Drilling of the femoral head is the method that can be applied to increase revascularization and thus enhance blood supply. Kong et al. [15] stated that multiple drilling of the femoral head accelerated revascularization in their study on immature piglets. Wang et al. [17] stated that adding adipose tissue-derived stem cells and BMP -2 to drilling could induce new bone formation and prevent the collapse of the femoral head epiphysis in the early stages of femoral head necrosis. Herrera Soto and Price [16], in addition to shelf acetabuloplasty in the treatment of juvenile avascular necrosis of the femoral head allow removal of the necrotic segment and improve vascularization in the affected area. We think that accelerating the revascularization by drilling the necrotic area in the treatment of LCPD will shorten the course of the disease, thus minimizing the deformity that may occur in the femoral head. In addition, decreasing the duration of necrosis and fragmentation periods will save more time for remodeling, so that a more spherical femoral head and congruent hip joint will be obtained. We strongly recommend performing the BEST procedure during the necrosis stage and/or before fragmentation to shorten the necrosis stage.

The main drawback of drilling given in the literature is the risk of permanent cessation of the growth plate. However, Bowen et al. [32] reported that premature physeal closure was observed in 23% of patients with LCPD, and it was more common in patients who did not undergo surgery. Park et al. [19] observed premature physeal closure in 21 (77.8%) of 27 unilateral LCPD patients they treated conservatively and stated that premature physeal closure was associated with the Herring class of the disease (more in Herring B/C and C) but not the Stulberg class. In other words, premature closure of the femoral head physis does not seem to cause inferior results of the disease. In summary, the main goal should be obtaining spherical femoral heads in which the limb can be shorter than the other extremity, rather than having equal leg lengths but with aspheric non-congruency. Seven of our patients (29.1%) had an average leg length discrepancy of 5.2 mm. This rate was well below the 77% premature physeal closure rate encountered in patients treated conservatively determined by Park et al. [19].

Skin traction is a conservative treatment method with some success in treating of LCPD. Wagenaar et al. [33] observed that patients treated with long-term traction achieved similar success to patients treated with femoral varus osteotomy. However, they stated that long-term application might cause problems due to muscle atrophy, osteopenia, negative social and psychological effects. Thus, it is not very suitable for today's conditions and can be applied for a short time by combined with other treatment methods. We recommend skin traction for four weeks to enhance osteogenesis and angiogenesis by distraction and secure the pelvic osteotomy fixation by its immobilization effect.

Synovitis of the affected hip joint is a common feature of LCPD, producing pain, loss of hip motion and chondrolysis. Additionally, medial and inferior seating of the excess synovial fluid in the joint may cause reflected pain on the medial knee by obturator nerve innervation. It may also cause a lateral shift of the femoral head in the acetabulum. Kamiya et al. [34] stated that the IL-6 level of the synovial fluid is significantly higher in LCPD, which may have significant effects on healing. In addition, Upasani et al. [35] stated that the increase in intraarticular pressure negatively affects blood flow to the femoral head in their study on animals. Thus, evacuation of the fluid with pathological content, which both causes an increase in the pressure in the joint and negatively affects the blood flow to the head, and has the potential to have a negative effect on healing, may contribute to the acceleration of healing. We also postulated that evacuation of the fluid lowers the pressure in the inferomedial pocket of the capsule, which further relieves the pain reflected in the knee and also helps increase the containment. Therefore, we include the evacuation of pathological fluid in the joint in our treatment protocol for its physical and chemical benefits.

Our study had some limitations. First, our study was a retrospective study with no control group. We had no chance to compare our method with any other treatment method. Second, the number of our patients was relatively low. This did not give an idea about which stage of the treatment would be more successful. Prospective studies with many patients are needed to make stronger recommendations.

In conclusion the BEST treatment method for LCPD is a combined procedure that addresses all pathologies of the disease at the same time. We recommend this combined treatment protocol (BEST procedure) for severely affected LCPD cases over six years of age and subluxation, especially in the higher lateral pillar class. We need a longer follow-up duration and larger numbers in our series to strengthen our conclusion for our method in LCPD treatment.

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

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Authors' contributions to the article

R.C., E.T., and C.O. constructed the main idea and hypothesis of the study. R.C. and C.O. developed the theory and arranged the material and method section. R.C. and E.T. have evaluated the data in the results section. Discussion section of the article written by R.C., E.T., and C.O.

R.C., and C.O. reviewed, corrected, and approved. In addition, all authors discussed the entire study and approved the final version.