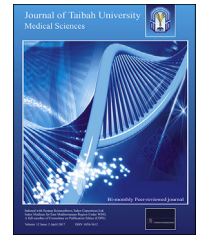




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Original Article

Effectiveness of surgically treated symptomatic plano-valgus deformity by the calcaneo stop procedure according to radiological, functional and gait parameters



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المخلص

أهداف البحث: إن نسبة الأقدام المسطحة في الأطفال والبالغين هي حوالي ٥٪. ويُنتج النوع المصاحب بالأعراض من الأقدام المسطحة المرنة شكوى ذاتية وموضوعية مثل اضطرابات المشية. ويُلبَّأ إلى التدخل الجراحي عند فشل العلاج التحفظي. وقد بينت الأبحاث أن إجراءات التقييد المفصلي توفر قدماً مستقرةً وتصحيحاً ممتناً، إلا أنه لم تُعم أي من الدراسات بتقييم فاعلية هذا الإجراء على معالم المشية.

طرق البحث: أُجريت عملية "إيقاف عظمة العقب" على ١٥ طفلاً لديهم أقداماً مسطحة مؤلمة (١٠ أولاد و٥ بنات) بمتوسط عمر ١٢ سنة و٦ أشهر. وتم تقييم الأشعة وتحليل المشية والقياسات الوظيفية لمعرفة التغيرات قبل وبعد العملية.

النتائج: تمت متابعة جميع الأطفال لمدة متوسطة ٤ سنوات و٦ أشهر. وأظهرت النتائج العامة للدراسة تحسناً ذا قيمة إحصائية لجميع المتغيرات الإشعاعية والقياسات الوظيفية ومدى الحركة التي تم التحقق منها في الدراسة. على الرغم من أن الدراسة أظهرت تحسناً في جميع المعالم الزمنية التي تم قياسها عند تحليل المشية، إلا أن النتائج لم تُظهر أي اختلافات ذات قيمة إحصائية في المتغيرات الحركية القياسية والحركية قبل وبعد الجراحة.

الاستنتاجات: على الرغم من عدم وجود تحسن ذي قيمة في المتغيرات الحركية القياسية والحركية التي تم التحقيق فيها، نستنتج بأن عملية "إيقاف عظمة العقب" هي عملية فعالة ويمكن الاعتماد عليها في علاج القدم المسطحة المرنة لدى الأطفال.

الكلمات المفتاحية: القدم المسطحة؛ المشية؛ ظاهرة حركية حيوية؛ الجراحة؛ طفرة حينية؛ عملية إيقاف عظمة العقب

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Abstract

Background: The incidence of flatfoot is approximately 5% in children and adults. The symptomatic form of a flexible flatfoot produces subjective and objective complaints, including gait disturbances. Surgical intervention is considered when conservative management fails. The arthroereisis procedure provides a stable foot and durable correction. However, to the best of our knowledge, no study has evaluated the effectiveness of this procedure on gait parameters.

Methods: Fifteen children with painful flatfeet (10 males; 5 females) with a mean age of 12 years and 6 months underwent the calcaneo stop procedure. Radiographic, gait analysis and functional measures were evaluated to assess the changes pre- and post-operatively.

Results: All children were followed up for a mean period of 4 years and 6 months. The overall results of the study exhibited statistically significant improvement for all radiographic variables, functional measures and range of motion investigated in the study ($p < 0.05$). Although the study showed improvement in all temporal parameters ($p < 0.05$) measured in the gait analysis, the results demonstrated there were no statistically significant differences in the kinematic and kinetic variables ($p > 0.05$) before and after surgery.

Conclusions: Although there was no significant improvement in the kinematic and kinetic variables

investigated, this study supports the calcaneo-stop procedure as a reliable and effective procedure for treating paediatric flexible flatfoot.

Keywords: Biomechanical phenomena; Flatfoot; Gait; Surgery mutation

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Introduction

Flatfoot is a developmental or acquired deformity that is progressive, and it is characterized by plantar medial rotation of the talus, a decrease in the medial arch height, and abduction of the forefoot.¹ The incidence of flat foot is approximately 5% in children and adults.^{2,3} Flexible paediatric flatfoot is one of the most common deformities of the human body.⁴ Flexible flatfoot is characterized by the normal architecture of the medial longitudinal arch during non-weight bearing and flattening of the arch during stance or weight bearing.^{5,6} Flexible flatfoot may be symptomatic or asymptomatic.⁵

Symptomatic forms of flexible flatfoot produce subjective and objective complaints, including pain along the medial side of the foot, sinus tarsi, leg, and knee. Flatfoot decreases endurance and leads to gait disturbances.⁵ Pronation of the subtalar joint during the propulsive phase of gait is mostly responsible for major deformities in adult life.⁷ Flatfoot may also lead to hallux valgus, metatarsalgia, tarsal tunnel syndrome, posterior tibial tendon dysfunction, and osteoarthritis of the subtalar and midtarsal joints.^{8,9} Flat foot is associated with bio-mechanical changes and leads to a general structural deformity of the lower extremity.¹⁰ Initial management for flatfoot includes activity modifications, orthoses,¹¹ foot exercises¹² and nonsteroidal anti-inflammatory medications. If conservative management fails, surgical intervention can be considered.^{13–15}

Surgical management of flatfoot can be grouped into the following three types: soft tissue, bone (osteotomies and arthrodesis) and arthroereisis.¹⁶ Soft tissue reconstruction of the flexible flatfoot is rarely successful as an isolated procedure and should always be combined with bony procedures or arthroereisis.¹⁷ Excellent results from the reconstructive bony procedures of flatfoot have been reported. However, the long-term results are not satisfactory.¹⁸ Arthrodesis (extra-articular subtalar arthrodesis for symptomatic plano-valgus feet and triple arthrodesis for failed surgical treatment) provides a stable foot and durable correction. However, this procedure transfers energy to the non-fused adjacent joints, which may lead to early arthritis.^{19,20}

Arthroereisis limits subtalar joint pronation through insertion of an implant or material into the sinus tarsi.^{21–23} The presence of an implant achieves correction by stimulating the proprioceptive foot receptors, allowing for normal subtalar joint motion,¹⁶ while blocking excessive movement. Different shapes and implant designs have been

proposed, including bone grafts, polyethylene, silicone, bioresorbable materials, and metallic implants.²⁴ Kellermann et al., 2011²⁵ reported better short-term outcomes for pedographic parameters using a screw through the sinus tarsi into the talus. Pavone et al., 2013²⁶ reported good results on clinical evaluation, podoscopic examination, and radiologic assessment in 242 patients who were treated using the calcaneo-stop procedure. In a meta-analysis of the literature regarding arthroereisis in children with a flexible flatfoot, Metcalfe and colleagues²⁷ found that the majority of the studies were case reports and retrospective case series (Levels III and IV evidence). Hence, the purpose of this study is to evaluate the effectiveness of this procedure in a prospective manner and to observe if significant improvements in radiographic parameters would result in gait changes, as measured by kinetic and kinematic variables.

Materials and Methods

All children who presented to our institute with painful flatfeet were considered for screening of inclusion and exclusion criteria. A clinical diagnosis of plano-valgus deformity was based on an increased valgus position at rest and during the tip toe standing test as well as restriction of dorsiflexion of the ankle joint in the neutral varus/valgus position. Inclusion criteria for the study were failure with conservative treatment procedures, which included foot exercises and medial heel and sole raise exercises with arch support for a period of one and half years to 2 years. Children were excluded if there were no adequate records for documentation of conservative management and if the flatfoot was secondary to joint hyperlaxity, dystrophy, post-traumatic, neurogenic, or neuromuscular disorders.

A total of 55 children (92 feet) reported they had flatfeet during the study period (Jan 2007–Oct 2010). Nineteen children had no documented evidence of conservative management and were referred for physiotherapy and orthotic management. Sixteen children did not meet the inclusion criteria or met some of the exclusion criteria. The parents of 3 children opted against surgery. Two children were lost to follow-up. Fifteen children (10 males and 5 females) (25 feet) (mean age 12 years and 6 months) fully participated in and completed the study (Table 1). The children were followed for a mean duration of 4 years and 6 months (range of 2 years and 8 months to 6 years and 3 months). The institute ethical committee provided ethical clearance for the study and all the primary caregivers of the children provided informed consent.

Outcome measures

Passive ankle and subtalar range of motion (ROM) using a 360-degree goniometer

A 360-degree plastic goniometer with 1-degree increments was used. The children were positioned on the bed for long sitting and reclined to approximately 45° with a pillow under the upper part of their lower legs to flex the knee to 20–30°. The angles were measured from the neutral (plantigrade) position (0°). The ankle ROM was measured by placing the stationary arm of the goniometer parallel to the longitudinal

Table 1: Mean age, height and weight of the 15 analysed children.

Sex	10 boys and 5 girls
Age	12 years and 6 months
Height (in cm)	127.4 ± 8.4
Weight (in kg)	33.4 ± 4.6

axis of the fibula, lining up with the fibula head and moveable arm parallel to the longitudinal axis of the 5th metatarsal. The axis of the goniometer was placed approximately 1.5 cm inferior to the lateral malleolus. The examiner passively pulled the foot towards dorsiflexion and the ROM was recorded by measuring the angle between the movable and stationary arms of the goniometer in degrees.

The subtalar ROM was measured by keeping the axis of the goniometer on the front of the ankle at the midpoint between the medial and lateral malleoli. The stationary arm of the goniometer was placed along the tibial crest and the moveable arm was kept in line with the 2nd metatarsal. The examiner then maximally inverted the heel, and the ROM was recorded by measuring the angle between the movable and stationary arms of the goniometer in degrees.

Visual analogue scale for foot and ankle (VASFA)

The VASFA consists of a 20-question questionnaire based on three different question categories (pain, $n = 4$ questions; function, $n = 11$; and other complaints, $n = 5$). The total value for the entire score, when all 20 questions were answered, ranged between 0 and 100 points. VASFA is a valid and reliable metric.²⁸

The American orthopaedic foot & ankle society score (AOFAS)

The AOFAS is the gold-standard score for the foot and ankle.²⁹ AOFAS is easy to apply and understand, and it can be applied to different kinds of injuries and treatments.³⁰

The Oxford ankle foot questionnaire for children (OxAFQ-C)

The OxAFQ-C³¹ is a self-report health status measure questionnaire for foot and ankle conditions that children (aged 5–16 years) can complete. The OxAFQ-C has 3 domains (a total of 15 items), Physical (6 items), School and Play (4 items) and Emotional (4 items). The three domain scores are reported separately and there is no total score.

Radiographic indices

The following angles were measured from weight bearing dorsoplantar (DP) and lateral foot radiographs:

Costa-Bartani angle

The angle created by the line formed from the lower point of the 5th metatarsal to the lower point of the calcaneocuboid joint, and the line formed from the lower point of the

calcaneocuboid joint to the lower point of the posterior calcaneal tuberosity in the lateral view.

Kite angle

The angle formed between the lines drawn down the axis of the talus and calcaneus measured on the loaded DP foot radiograph.

Calcaneal inclination angle

The angle between the calcaneal inclination axis and the supporting surface on a weight-bearing lateral foot radiograph.

Talar declination angle

The angle between the mid-talar axis and supporting surface on the weight-bearing lateral foot radiograph.

Talo-metatarsal angle (Meary's angle)

The angle between the line drawn from the centres of the longitudinal axes of the talus and the first metatarsal on the weight-bearing lateral foot radiograph.

Kinematic and kinetic analysis

Kinematic and kinetic data were collected with a six-camera motion capture system (BTS Smart-DX system, NY, USA; sampling rate: 200 Hz) and 1 force-plate (sensing area 1800 × 800 mm) during barefoot level walking. The analyse-space for the cameras was calibrated daily on the day of measurements by moving and rotating a rod with reflecting markers in the space that the children had walked. The analysis of foot movement during gait was performed according to the simple Helen Hayes protocol by an independent experienced assessor. The simple Helen Hayes protocol was selected because this protocol requires minimal patient preparation and fewer trajectories to track and edit the studied parameters.^{32,33} All children's sex, height (in cm) and weight (in kg) were noted. The following anthropometric measures were calculated: anterior superior iliac spine breadth, pelvis depth, leg length, knee diameter and malleolus width. The markers were applied while the patient held an orthostatic position. The Markers were secured at anatomical landmarks that determine embedded axes for segments. For the analysis, 15 numbers of 10-mm lower extremity markers were attached; 2 markers were attached to the anterior superior iliac spines (one right and one left), 1 marker to the second sacral vertebra, 1 marker to each of the lateral femoral condyles, one each on the lateral bar that was securely attached to the thigh using an adaptable strap, 1 marker to each lateral malleolus, one fixed on a rigid bar attached to the side of the shank using an adaptable strap, 1 marker each in the space between the heads of the second and third metatarsals, and one on each heel.

All participants were instructed to perform 2 different tasks. One practice trial was allowed before the actual trial. The children were required to stand in a normal, relaxed upright position, standing in a shoulder-width stance for 3–5 s on the force platform. This ensured that the participants' feet were aligned, which avoided having one foot in a more anterior or posterior position than the other (Figure 1).

The children were instructed to walk along a nine-metre walkway at a comfortable walking speed. The markers

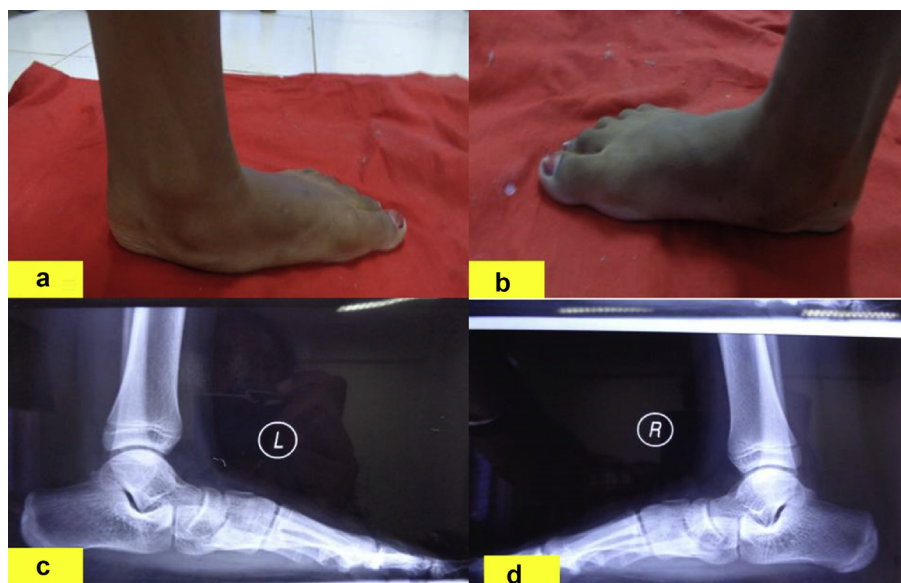


Figure 1: Pre-operative foot of a patient with pes-planovalgus (a and b), Pre-operative X-ray of the same patient with pes-planovalgus (c and d).

placed on the subject were clearly within the field of view of the cameras during the entire acquisition. It was ensured that all children hit the force-plates once with the measured foot during walking. The children were instructed to hit the force-plate spontaneously, and they were not forced to achieve this result. The force plates were used to determine the resulting moments and power in the subject's hip, knee and ankle joints for the measured leg. The protocol requires a single acquisition while the subject performs the standing task and at least one acquisition during a walking sequence. Although both legs of each participant were measured, data from only one leg were analysed (the affected ones for unilateral feet and the more affected one (maximum heel valgus in standing position, as determined by a physiotherapist) in bilateral cases).

Procedure

Operative procedure

The children were placed in the supine position under general anaesthesia with a tight tourniquet with the foot was internally rotated. An incision of 2 cm was made under the skin lines on the lateral aspect of the sinus tarsi (Figure 2a). Soft tissue dissection was performed, taking care to avoid the sural nerve. A guidewire was vertically inserted into the calcaneus from the superior to the inferior aspect opposite of the sinus tarsi after reduction of the subtalar eversion under fluoroscopic control (Figure 2c). This was followed by drilling with a 3.2-mm bit and insertion of a 6.5-mm cortical, stainless self-tapping screw (8 mm head) with a length of 30–35 mm (Figure 2d). The surgeon ensured that the screw head impinged against the lateral aspect of the talus, preventing eversion at the subtalar joint. The dorsiflexion of the foot was evaluated with the knee extended after appropriate placement of the screw had

been radiographically verified. Concomitant lengthening of the Achilles tendon was performed if dorsiflexion was not possible for at least to 5–10° after surgery. The patients were allowed full weight bearing within 3 days. Three weeks of cast immobilization was applied in patients in whom Achilles tendon lengthening had been performed. The screws were removed an average of 3 years after surgery following growth arrest of the foot.

Statistical analysis

Data were analysed with SPSS 16.0 (IBM Corp., Chicago, Illinois, USA). The paired Student's *t* test was used to compare the pre-operative outcome assessed by the physiotherapist under supervision of a surgeon at 6 months before surgery, 3 months before surgery and the day before surgery; also, post-operative clinical, radiographic and gait variables outcomes were measured at 3, 6 and 12 months and then once every year. The *p* value was set at 0.05.

Results

Only those children who complained of severe pain and discomfort in the ankle and foot were considered for surgery. All children reported a satisfactory clinical result; the valgus deformity of the hindfoot was corrected, a longitudinal arch was created and the prominence of the talar head was reduced during weight-bearing activities (Figure 3a,b,c). Children and their caretakers were pleased with the outcomes of surgery (Figure 4a and b). Seven feet (28%) required concomitant lengthening of the Achilles tendon. None of the children required post-operative braces.

Table 2 lists the baseline and follow-up scores of all radiographic variables, functional measures and the range of motion investigated in the study. The overall results of the study showed statistically significant differences between the

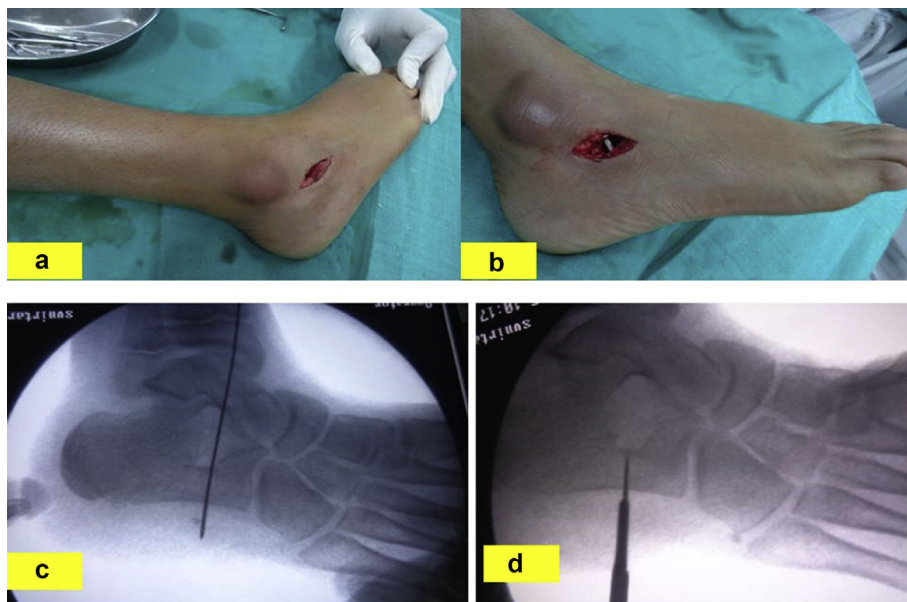


Figure 2: a & b: 6.5-mm cortical stainless self-tapping screw in situ; c & d: Calcaneo-stop procedure with a 6.5-mm cortical, stainless self-tapping screw.

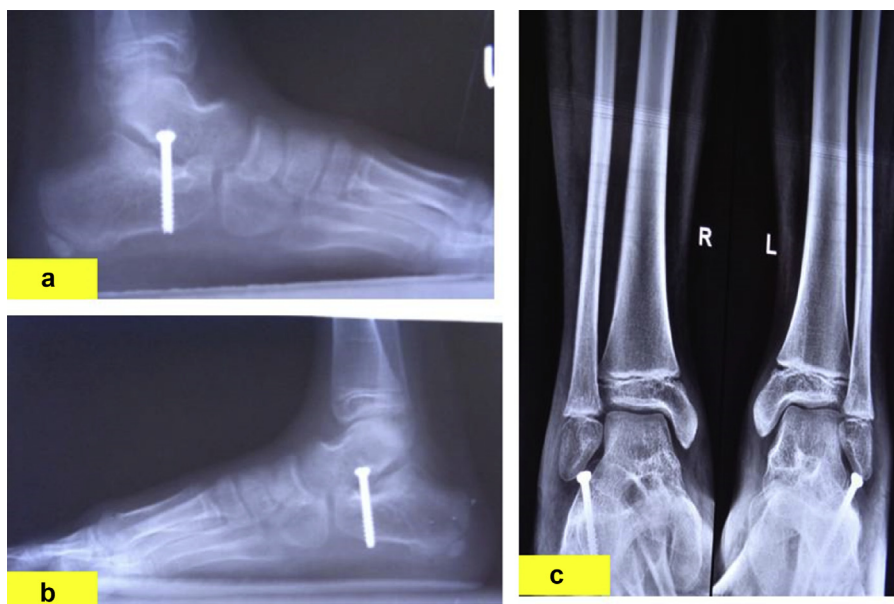


Figure 3: a, b & c Post-operative X-ray of the same patient.



Figure 4: b: Foot of a participant after surgery.

Table 2: Pre-operative and post-operative outcome.

Outcome measures	Pre-operative (mean \pm SD)	Post-operative (mean \pm SD)	t value	p value
Radiographic angles				
Costa Bartani angle	147.88 (3.12)	135.48 (2.69)	16.30	0.00
Kite angle	30.92 (1.42)	23.07 (0.94)	22.58	0.00
Calcaneal inclination angle	14.87 (0.56)	21.60 (1.00)	39.44	0.00
Talar declination angle	44.32 (2.12)	24.65 (1.12)	46.45	0.00
Talo-metatarsal angle	32.70 (0.43)	18.0 (0.41)	115.62	0.00
Functional scales				
VASFA	72.86 (7.33)	55.70 (7.16)	7.82	0.00
AOFAS	35.44 (8.31)	62.68 (8.11)	19.03	0.00
OAFQC				
Physical	73.20 (6.71)	82.79 (5.59)	12.39	0.00
Sports	70.24 (5.79)	80.16 (3.84)	9.51	0.00
Emotional	72.33 (7.26)	82.64 (7.08)	5.71	0.00
Range of motion				
Ankle – dorsiflexion	11.88 (2.22)	21.64 (2.81)	12.09	0.00
Subtalar-supination	6.72 (1.74)	14.48 (2.84)	11.73	0.00

pre-operative and post-operative measurements for each radiographic and functional variable ($p < 0.05$) (Table 2). Gait analysis showed improvement in the temporal gait parameters (Table 3). However, the analysis showed no statistically significant differences in the kinematic and kinetic variables ($p > 0.05$) before and after surgery (Table 4). One patient had a complication of a loosened screw that caused pain and restricted motion of the subtalar joint. The implant was removed and arthroereisis was performed using a new screw. Other minor complications included ($n = 3$) local symptoms at the incision and delayed wound healing as well as contracture of the peroneal muscles (treated with physiotherapy).

Discussion

The results of the study showed that the calcaneo stop procedure has good outcomes for all measured parameters,

except gait analysis. The procedure showed significant positive changes both clinically and radiographically (Figure 3a,b,c).

It has been estimated that 63% of children with flatfeet have functional impairment.³⁴ This impairment has been related to early muscle fatigue and foot-and-ankle-complex instability.³⁵ Numerous treatment options have been recommended, but no single procedure shows adequate correction and resolution of the patient's symptoms.

Arthroereisis procedures are designed to limit subtalar joint motion and improve the weight-bearing position of the foot by placing a motion-blocking implant into the sinus tarsi. The surgical technique adopted in this study was a simple, small lateral incision at the sinus tarsi, as described by Giannini et al., 2001³⁶ and Carranza et al., 2000.³⁷ This procedure is less invasive than the combined medial and lateral approaches described by Viladot, 1975.³⁸ The results are similar to those reported by Brancheau et al., 2012³⁹ Jerosch et al., 2009⁴⁰ and Pavone et al., 2001.²⁶

The significant improvement in all radiological parameters may be explained by the biomechanics behind the surgery. In flatfoot deformities, the intersection site of the talus and first metatarsal axis mostly occur at the talonavicular joint. If Paley's concept of the centre of rotation of angulation of the deformity is applied to the foot, the calcaneo-stop procedure acts at the centre of rotation of angulation of the deformity.⁴¹ The insertion of the implant into the sinus tarsi restricts the motion between the talus and calcaneus, restricting valgus deformity. By orienting the calcaneus more vertically beneath the talus and ankle joint, the subtalar joint axis is altered, correcting the hindfoot alignment and mid and forefoot deformities. When the calcaneus and cuboid are inverted, the talus dorsiflexes and externally rotate and the navicular inverts, confirming the supinatory effects of the subtalar arthroereisis.⁴² However, the improvement in static radiographic measures did not alter the movement patterns associated with gait.

Table 3: Temporal parameters.

Measures	Pre-operative	Post-operative	p value
Stride time (in seconds)	0.82 (0.34)	1.07 (0.6)	0.00
Stance time (in seconds)	0.59 (0.02)	0.67 (0.12)	0.02
Swing time (in seconds)	0.3 (0.09)	0.39 (0.04)	0.03
Stance phase (as a percentage)	61.42 (27.11)	58.35 (4.13)	0.01
Swing phase (as a percentage)	26.75 (14.86)	45.64 (2.18)	0.00
Double support phase (as a percentage)	17.56 (16.27)	19.21 (12.4)	0.03
Mean velocity (in metres/second)	0.8 (0.5)	1.3 (0.1)	0.00
Mean velocity (in percentage height/seconds)	49.56 (33.81)	79.0 (6.72)	0.00
Cadence (steps/min)	90.34 (57.2)	118.65 (20.30)	0.00

Table 4: Values of the joint rotations obtained on gait analysis.

	Pre-operative	Post-operative	<i>p</i> value
<i>Values for the joint rotations obtained with gait analysis</i>			
Rotations (in degrees)			
Pelvic obliquity	4.4 (1)	4.2 (1.1)	0.08
Pelvic tilt	10.9 (0.9)	12.4 (1.5)	0.54
Pelvic rotation	8.3 (1)	8.4 (1.2)	0.05
Hip flexion-extension	10.1 (2.9)	9.8 (2.8)	0.1
Knee flexion-extension	8.0 (2.6)	8.3 (2.4)	0.23
Ankle dorsi-plantar flexion	9.9 (1.5)	11.3 (1.4)	0.054
Hip abduction/adduction	-2.8 (1.3)	- 2.1 (0.4)	0.034
Hip rotation	6.9 (1.9)	7.8 (1.6)	0.00
Foot progression	2.3 (0.3)	2.1 (0.2)	0.051
<i>Values for the joint moments obtained with gait analysis</i>			
Moments (in % BW X H)			
Hip flexion-extension	-1.2 (0.8)	-1 (0.3)	0.87
Knee flexion-extension	-0.5 (0.03)	-0.5 (0.02)	1.0
Ankle dorsi-plantar flexion	2.7 (0.5)	2.9 (1.1)	0.06
Hip abduction/adduction	3.6 (0.2)	3.5 (0.9)	0.9
Hip rotation	0.2 (0.01)	0.1 (0.08)	1.0

BW = body weight; H = height.

The results showed changes in the temporal parameters with relatively little change in the collected kinematic and kinetic data before and after surgery. The absence of an effect of procedure on the evaluated kinematic variables may be hypothesized to be because the subtalar joint arthroereisis does not alter the normal closed kinetic chain mechanics.⁴² The mean velocity and cadence improved to near normal values after the surgery, which may be due to the reduction in pain following surgery. Twomey, 2010⁴³ showed that the kinematic differences between the normal arched and low arched feet in children between 9 and 12 years old, using the Heidelberg foot measurement method during walking, were relatively small. Gait analysis is also prone to human errors because the interpretation relies heavily on the skill of the assessors in placing the markers.⁴⁴ Furthermore, there is uncertainty about the capacity of the Helen Hayes marker set to accurately capture ankle motion.³³

The reduction in pain and improvement in function, as observed in this procedure, may be attributed to the neuro proprioceptive⁴⁵⁻⁴⁷ role of this surgical procedure. The calcaneo-stop procedure is completely extra-articular, resulting in fewer biomechanical stress symptoms.⁴⁵ Needleman suggested that the improvement seen in functional scores and symptom release after the procedure are biomechanical in nature.⁶

Although the surgical procedure treated the biomechanical aspects, the muscle activities were not studied. Furthermore, recruitment of patients with bilateral and unilateral flatfoot might have influenced the gait variables. Based on prior reports, the muscle tone and stiffness of the tibialis anterior and medial gastrocnemius muscle vary between the bilateral and unilateral flatfoot population.⁴⁸ The study used one marker on the foot and one on the ankle, which made it difficult to evaluate frontal and transverse plane motion as well as to de-identify multiple foot joints. Additionally, there was no control group for comparison of our outcomes. The potential biases related to measuring the radiographic angles and the biases related

to identifying the potentially eligible patients may not be ruled out.

Future studies should consider a comparative gait analysis of flat footed children that is corrected by this surgical approach and normal children by simultaneously evaluating clinical variables, joint kinematics, muscle activations, ground reaction forces and net joint moments to fully evaluate the effectiveness of this procedure.

Conclusions

The calcaneo-stop procedure is a simple, reliable, effective and minimally invasive procedure for treating paediatric flexible flatfoot. The lack of improvement in gait parameters may be attributed to the foot modelling technique used in the study rather than the operating technique. We recommend further studies use a multi-segment kinematic model, such as the Oxford Foot Model.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

SPD conceived, designed study, provided research materials and wrote the initial draft for the article. PBD conducted research and collected, organized data and provided logistic support and wrote the final draft of the article. GS documented all the data generated from the experiments, helped in the design of the study and final drafting of the article. MCS analyzed and interpreted data. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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