

An evaluation of Treatment of Congenital Talipes Equino Varus by Ponseti method at Agartala Government Medical College

Dr. Nirmal Debbarma¹, Dr. Ajit Tudu², Dr. Bhupes Sil³, Dr. Tapan Kumar Das⁴

¹Junior Resident (Academic), Department of Orthopaedics, Agartala Government Medical College and Govind Ballabh Pant, Hospital, Agartala, Tripura

²Junior Resident (Academic), Department of Orthopaedics, Agartala Government Medical College and Govind Ballabh Pant, Hospital, Agartala, Tripura

³Associate Professor, Department of Orthopaedics, Agartala Government Medical College and Govind Ballabh Pant, Hospital, Agartala, Tripura

⁴Professor, Department of Orthopaedics, Agartala Government Medical College and Govind Ballabh Pant, Hospital, Agartala, Tripura

Abstract: Background: The Congenital Talipes Equino Varus (CTEV) or Clubfoot is a common congenital deformity of the foot. The incidence of Clubfoot is 1 per 1,000 live births with approximately 50% having bilateral. The ratio of male and female is 2.5:1. In unilateral cases, there was a right sided predominance. The real cause of Congenital Talipes Equino Varus is still unknown. The aim of treatment is to obtain anatomically and functionally normal feet. Clubfoot has been treated by serial manipulation and plaster casts. The disadvantages of surgery are high complication and recurrence. Aim: The aim of this study is to evaluate the results of Ponseti technique in the management of Clubfoot. Objectives: The objective is to identify the cases of Club foot & to assess the deformity correction by Pirani scoring system. Place and Duration: Study were done in Orthopaedics OPD at Agartala Government Medical College & GBP Hospital Between December 2014 to June 2016. Material & Methods: Between December 2014 to June 2016, total 63 cases of Clubfoot of age upto 1(One year) were enrolled in Orthopaedics OPD at Agartala Government Medical College & GBP Hospital. Among these, 3 cases were syndromic and excluded. Others 7 cases were drop out during the period of treatment could not be followed up. Results: In our study Ponseti method proved successful, with all the children achieving an excellent outcome when evaluated by Pirani scoring system. Conclusion: Ponseti method is effective in correction of idiopathic clubfoot in children up to 12 months of age.

Keywords: Club Foot, 50% Bilateral, Pirani Scoring System, Ponseti Method(Serial Manipulation And Plaster Cast).

1. Introduction

The Congenital Talipes Equino Varus (CTEV) or Clubfoot is a common congenital deformity of the foot. The term Talipes Equinovarus is derived from Latin: Talus (ankle) and Pes (foot); Equinus : "Horse like" (the heel in plantar flexion) and Varus : inverted and adducted¹. The incidence of clubfoot varies widely with race and sex². The overall estimated incidence of Congenital Talipes Equino Varus is 1 per 1,000 live births with approximately 50% having bilateral Clubfeet³. First degree relatives have 20 times higher incidence than expected incidence in general public⁴. The ratio of male and female is 2.5:1 and 24.4% of affected individual have a family history of Congenital Talipes Equino Varus⁵. In those with unilateral deformity, there was a right sided predominance⁶.

The real cause of Congenital Talipes Equino Varus is still unknown. However, there are some key factors including genetic factors, histological anomalies, vascular anomalies and intrauterine factors which give some clue about the aetiology⁷. Some cases are associated with neuromuscular disease, chromosomal abnormalities, different syndromes or extrinsic causes, others occur in otherwise normal infants and are classified as Idiopathic Congenital Talipes Equino Varus. The later is a common but still not fully understood disorder. Diagnosis is mainly based on clinical evidence^{8,9}. It

is always recognizable at birth but with recent advances it is now frequently diagnosed at 18 to 20 weeks of gestation by ultrasound¹⁰.

The aim of treatment is to obtain anatomically and functionally normal feet in all patients¹¹. Clubfoot has been treated by manipulations, plaster casts, strapping, splintage and surgery¹². Among these, Kite's method of manipulation & casting has been popular among Orthopaedic surgeons. Recently Ponseti's method of manipulation & serial casting has been shown to be successful reporting good results at short term follow up in infants where treatment was started within first few months of life¹³⁻¹⁵. There is nearly universal agreement that the initial treatment of the Clubfoot should be non-operative regardless of the severity of the deformity. If there is no improvement then most of the Surgeons prefer postero-medial release (PMR) of the soft tissues. The primary disadvantages of postero- medial release are high complication and recurrence (13-50%)¹⁶⁻²². Most of the authors have concluded that extensive surgery is not the right approach to management of Congenital Talipes Equino Varus¹⁶⁻²². Conservative treatment of Clubfoot is an accepted practice and has been reported to result in good correction ranging in 50-90% of cases¹⁴. Treatment should be started early to ensure better outcomes allowing optimal growth of bone (particularly talus) and maintenance of joint mobility¹⁸⁻²².

2. Methodology

Ethical approval was taken from Institutional Ethical Committee, Agartala Government Medical College. 60 cases entered during december 2014 to june, 2016 after explaining the study protocol and possible necessity for Achilles tenotomy and foot abduction Orthosis till the age of 4 years. Appropriate Informed consent was taken from the parents. All details of the parents and baby was recorded. 53 children completed serial castings with or without Achilles tenotomy and were given foot abduction Orthosis and were followed up. Outcomes of management of Congenital Talipes Equino Varus of the baby were assessed by Pirani score system.

3. Results

63 cases were enrolled at out-patient department of Orthopaedics between the period December 2014 to June, 2016. Among these 63 cases 3 cases were Syndromic and excluded from the study. 60 cases were Idiopathic variety. Out of these 60; 53 cases could be followed, the other 7 cases could not be followed due to drop out and were only used for epidemiological data.

Table 1: Total number of cases registered during the study period

Type	No. of Cases	Percentage
Idiopathic	60	95%
Syndromic	03	5%

Table 2: Showing ratio of male and female

Sex	No. Of cases	Percentage
Male	38	63%
Female	22	37%

Table 3: Side Distribution

Involvement	No. of cases	Percentage
Bilateral	29	48%
Unilateral	31	52%

Table 4: Ratio of right and left side

Side	No. of cases	Percentage
Right	21	68%
Left	10	32%

Table 5: Family History

History	No. of cases	Percentage
Positive	2	3.3 %
Negative	58	96.7%

Table 6: Age Distribution

Age group	No of cases	Percentage
Day 1 - 3 months	35	59%
4-6 months	11	18%
7-9 months	6	10%
10-12 months	8	13%
Total	60	100%

Table 7: Showing distribution of age in relation to Pirani score and casts

Age Group	No. of feet	Mean Pirani score	Mean cast
Day1-3 months	47	4.79	6
4-6 months	17	5	8.5
7-9 months	7	4.35	8.28
10-12 months	8	3.87	9.75
Total	79	4.5	

Table 8: Showing number of tenotomy required

Tenotomy	No. of feet	Percentage
Yes	44	56%
No	35	44%

Table 9: Age- wise Tenotomy

Age Group	Tenotomy	Percentage
Day 1 to 3 months	18	38.2%
4 to 6 months	13	76.4%
7 to 9 months	5	71.4%
10 to 12 months	8	100%

Table 10: Relationship of initial Pirani score with Pirani score at 3rd months, 6 months and 1 year follow up

Age Group	Mean initial Pirani Score	Mean Pirani Score at 3 rd months Follow up	Mean Pirani Score at 6 th months follow up	Mean pirani score at 1 year follow up
Day 1 to 3 months	4.79	0	0	0
4 to 6 months	5	0	0	0
7 to 9 months	4.35	0	0	0
10to 12 months	3.87	0.18	0.37	0
Total	4.5	0.045	0.09	0

Table 11: Showing complication

Complication	No. of cases	Percentage
Skin abrasion	7	13.13%
Skin blister	0	0%
Slippage of cast	2	3.77%

Table 12: Showing outcome

Results	No. of feet	Percentage
Excellent	79	100%
Good	0	0
Poor	0	0

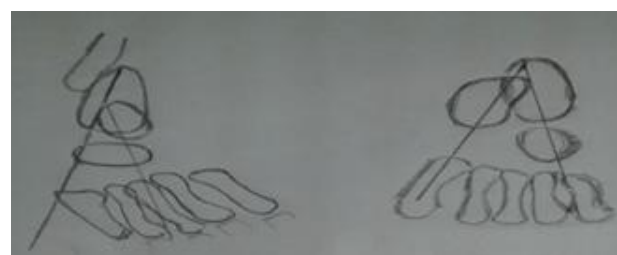


Figure: Showing- AP view of right Clubfoot with decrease in talo-calcaneal angle (left) & Talo-calcaneal angle of normal left foot (right)



Figure: Initial clubfoot deformity (Rt Foot)



Figure: After final cast (Rt Foot)

4. Discussion

Non-surgical management of clubfoot is the preferred method of initial management of the deformity in all over the world. Ponseti's method has been shown to be an effective method for the treatment which includes serial corrective manipulation a specific technique of cast application, a possible percutaneous tenotomy and a specific brace.

Recently, several studies of the Ponseti method have shown excellent results and has become accepted because extensive open surgery is commonly associated with long term stiffness and weakness which is avoided by the Ponseti technique. However, the literature contains primarily short-term follow up studies while long term follow up are scarce. The present study evaluates the efficacy of Ponseti method in children up to 1 year age.

Patient Profile: 53 children with Idiopathic Congenital Clubfoot completed the study from December 2014 to June, 2016. Total no of clubfoot was 79. All the patients were of age 1 week -12 months at initial casting. Mean age of the group was 3.95 months. Several authors have studied whether the initial age at presentation impacts the result of treatment. Abdelgawad et al²⁶ reported a 6.6% failure rate in patients who presented late for treatment (mean age, 36.3 weeks). Other report have suggested age at presentation does not affect the end result of treatment. Morcuende et al²⁵ had retrospectively analyzed the records of 157 patients (256 clubfeet). These were from the period 1991-2001 (11 years). Although the mean age of the children of this study has not been mentioned, 81% of children were younger than 6 months and 29% were older than 6 months. Herzenberg et al conducted a study with 27 patients, all under age of 3 months, undergoing Ponseti method of casting. This was compared to a control group of 27 patients who underwent conventional method of casting. In the study conducted by Lehman et al²⁴, 30 children 45 feet were treated by Ponseti method. The mean age at presentation was 8.2 weeks (range 0.5-40 weeks). This study divided the patients into two groups based on the success of treatment – group 1: who were given foot abduction orthosis after successful treatment and group 2: who had unsuccessful casting. The mean age of group 1 was 8.2 weeks and group 2 was 34 weeks. All 17 patients who presented for treatment after walking age achieved full correction in the Lourenco et al²⁷ work, and good results were achieved in a previous study that consisted of babies whose average age was 5 months. We found no relation between final outcome and patients age at presentation, but an increased number of casting was needed if the age of the children increased at the time of initiation of casts. There were 38 males and 22 females in the present study and male: female ratio is 1.7:1. Morcuende et al²⁵ reported a male: female ratio 2.13:1. Ponseti found the incidence six times higher among males²⁸. The United Kingdom talipes study²⁹ showed a male: female ratio 2.3:1. Lochmiller et al⁵ showed that CTEV occurs more often in males than in females. The male preponderance found in this study is in agreement with other studies. 29 cases were bilateral clubfeet (48%) and 31 had unilateral deformity. Among unilateral cases 21(68%) had right sided and 10 cases (32%) had left sided deformity. In Morcuende et al²⁵ study, 99 out of 157(66.1%) were bilateral clubfeet and 58 (36.95%) were unilateral. In lehman et al²⁴ series, the distribution of unilateral and bilateral cases was equal. In Wynne et al³⁰ series 50-70% of cases are bilateral. The right foot was more affected in our study. In the study of Boo and Ong³¹ left foot was more commonly affected. In contrast, Byron and Wallander³²⁻³³ reported right unilateral are more common.

Antenatal History: 55 patients, 92% were born by vaginal delivery and 5 cases by caesarean section. The reason for caesarean section was maternal pregnancy induced hypertension in one cases and breech delivery in 2 cases. Hippocrates had suggested that the foot held in equinus position by the external uterine compression and oligo-hydromnios. However Turco²³ had refuted this view and suggested that it is unlikely that such increase pressure would repeatedly produce the same deformity, especially

when there is plenty of room in the uterus at the time that a clubfoot develops (first trimester). In our study no any definitive conclusions to be drawn due to small sample size. The finding from this study shows that 60 cases were Idiopathic while 3 cases were Syndromic. This is similar to findings by Zosia³⁴ that Idiopathic CTEV is by far the most common. Tachdjian³⁵, reported that clubfoot affects both siblings in 32.5% of monozygotic twin but in only 29% of dizygotic twin. A history of a relative having idiopathic CTEV is common although the heritability varies between populations. Only 2 cases had a family history of CTEV in this study. The mean age at the initiation of treatment for 53 patients (79 feet) was 3.95 months; range 1 week to 12 months. The number of casts per foot in our study was 3 to 12 (average 8.1). In a series Ponseti et al³⁶⁻³⁷, the number of casts per foot was five to ten (average 7.6). In another study by Laaveg et al³⁸, the mean no of casts during their treatment was seven. The mean initial Pirani score was 4.5(out of maximum score 6). After full correction the final score was found to be 0 and the mean change of score was found to be 4.5. The mean value of Pirani score at three months follow up was 0.045. At the six months follow up, the Pirani score was 0.09 signifying mild increase in deformity. At 12 months it was 0. In Lehman series²⁴, Pirani score changed from 4.6 to 0.6 in mean 5.3 casts. The mean casts in our study is higher. In our series, tenotomy was required in 56% cases(38.2% of cases of day 1to 3 months age group; 76.4% cases of 4-6 months ; 71.4% cases of 7-9 months age and 100% cases of 10-12 months age group,) this means that tenotomy was required in those patients whose age was more at the time of initial treatment. Porecha et al³⁹ performed tenotomy in 97% of cases while Bor et al⁴⁰ performed tenotomy in 92.3%. In the retrospective study by Morcuende et al²⁵, correction was obtained in 98% cases. Corrcion was obtained in up to 7 casts. 90% of patients required 5 casts for correction. Tendoachilles tenotomy was done in 86% of the cases. The 100% correction obtained in our study may be due to smaller size sample (n= 79) compared to Morcuende et al study (n=256). Another factor that might have played role is that the end point for casting in our study was correction of the deformity irrespective of the number of casts taken to achieve the same. The number of casts required for correction ranged from 3 to 12. The mean number of casts required was 8.1 casts which is higher as compared to Morcuende series. An increase in deformity was noted by Pirani scoring. Increase in deformity, relapse occurred in 2 feet. These relapsed feet was corrected by corrective casts. In the relapse group, the mean age was 12 months while the mean age for the entire study was 3.95 months. Thus, in our study, age at initiation of treatment was found to be a risk factor for relapse. In this group, parents reported irregular use of the foot abduction orthosis. The real impact of this fact was not quantitatively assessed but might have contributed significantly to relapse of the deformity. Relapse appears related to non-compliance in wearing the orthosis, since all of these cases reported decreased duration of orthosis wear per day. In Lehman et al²⁴ study, 27 out of 38 feet were compliant with the orthosis and had good results at three months follow up. He found that the 11 feet of the non-compliant patients had good results as well at three months follow –up. In the retrospective study of Morcuende et al²⁵, clubfoot correction was obtained in 253 out of 256 feet(98%).Of the patients who had initial

successful correction, deformity relapsed in 10%.He found that this relapse was not related significantly to age at presentation, previous unsuccessful treatment , or the number of casts required for correction. He found that relapse were associated with non-compliance with foot abduction brace. Non-compliance was associated with a 17 times greater odds of relapse compared with compliance. The more the age at the time of initiation of treatment may need more number of casts for full correction and have a higher tendency for recurrence. Another factor contributing to the recurrence is the noncompliance with the use of foot abduction. Long –term studies are needed to follow the patients and to determine incidence of recurrence. In all the cases groin-toe casts were applied in our study. There were 7 cases of abrasion as a result of casting. This was due to inadequate cast padding. Healing of these abrasion can be aided by application of neomycin powder. Cast slippage occurred in 2 cases is due to loose cast and flexion at the knee was suboptimal. This can be prevented by meticulously adhering to the details of casting. No major bleeding occurred from tenotomy site.

In our study Ponseti method proved successful, with 100% of cases (79 Clubfoot) achieving an excellent to good outcome when evaluated by the Pirani scoring system. Porecha et al reported an excellent to good outcome in 86.56% of cases. In a report by Bor et al, the Ponseti method proved largely successful, with 89.2% achieving a good outcome. Ippolito et al compared babies treated with different protocols (Ponseti and Marino-Zuco method). In Ponseti group, 785 of the feet achieved excellent or good results compare with only 43% in the Non-Ponseti group.

From this study it appears that CTEV in children upto 1 year can be successfully corrected by Ponseti method. This correction can be achieved with larger than usual number of casts and with tendoachilles tenotomy. Persisting with casting in spite of slow correction may be successful up to 12 casts. Higher age of the patient necessarily does not mean that the deformity is resistant to correction but may necessitate a more prolong casting period. Tendoachilles tenotomy can be done safely without any adverse effect.

5. Conclusion

Ponseti method is effective in correction of idiopathic clubfoot in children up to 12 months of age. The number of casts needed for correction higher in older age groups. Persisting with castings despite slow correction yielded correction in upto12 casts. Higher age of the patient may not necessarily mean that the deformity is resistant to correction but may necessitate a more prolonged casting period. Tendoachilles tenotomy may be needed in a significant number of these cases. Tendoachilles tenotomy can be safely done up to 12 months of age with no apparent adverse effect apparent at three-month follow-up. Relapse of deformity was found to be more related to the non-compliance to foot abduction orthosis and the age of the patient at the time of initiation of casts. Strict adherence to the casting technique helps in successful correction. Compliance on the parents part in the use of foot abduction orthosis is essential to prevent the relapse of deformity. The most common cast-

related complications were skin abrasions and cast slippage due to loosening and these respond well to simple treatment.

6. Acknowledgement

We want to thank patients for their co-operation.

References

- [1] Strach E.H. Clubfoot Through the centuries. *Prog Pediatr Surg*. 1986; 20:215-237.
- [2] Cummings R.J. Lovell W.W. Current Concepts Review: Operative Treatment of Congenital Idiopathic Clubfoot. *J. Bone Joint Surg.*1988;76A(7):1108-1112.
- [3] Dobbs MB,Cummings R.J. Factors predictive of outcome after use of Ponseti method for the treatment of idiopathic Clubfeet. *J Bone Surg Am*2004; 86(1):22-7.
- [4] Mahboob G. Management of Congenital Talipes Equino varus (CTEV) at Jinnah postgraduate medical center,Karachi *J Pakistan Orthop Assoc*: 1994;10:75-7.
- [5] Lochmiller C.L,Johnston D, Scott A, Risman M, Hecht J.T. Genetic epidemiology study of Idiopathic Talipes Equino Varus. *Am J Med Genet* 1998;79:90-96.
- [6] Tachdjian M.O. *The Child Foot*. W.B.Saunders. Philadelphia.1985;pp.139-239.
- [7] Cummings R.J, Davidson R.S, Armstrong P.F. Congenital Clubfoot. *J Bone Joint Surg Am* 2002; 84-A: 290-308.
- [8] Wallander H, hovelius L, Michaelsson K. Incidence of Congenital clubfoot in Sweden. *Acta Orthop* 2006;77:847-852.
- [9] De Andrade M, Bonholtz J.S, Amos CL, Lochmiller C, Scott A, Risman M,Hecht J.T. Segregation analysis of idiopathic talipes equinovarus in Texan population.*Am J Med Genet* 1998;79:97-102.
- [10] Wainwright A.M, Auld T, Benson MK, Theologis TN. The classification of Congenital Talipes Equino Varus.*Journal of bone and Joint Surgery, British Volume*.2002;84(7):1020-4.
- [11] Lykissas MG, Crawford AH, Eismann EA, Tamai J. Ponseti method compared with soft tissue release for the management of Clubfoot: A meta analysis study. *World J Orthop* 2013 Jul 18;4(3):144-153.
- [12] Dietz FR, Ponseti IV, Bulkwalter JA .Morphometric study of Clubfoot tendon sheaths. *J Pediatr Orthop*.1985;5:577-81.
- [13] Kite JH. Nonoperative treatment ofCongenitalClubfoot.*Clin.Orthop*.1972;84:29-38.
- [14] Ponseti IV, Treatment of Congenital Clubfoot. *J Bone Joint Surg* 1992;74A:448-454.
- [15] Ponseti IV. Current concepts; common error in the treatment of Congenital Clubfoot.*Int Orthop* 1997;21:137-141.
- [16] Adegbehingbe O, Ogini LM, Ogundele OJ, Ariyibi AL, Abiola PO, Ojo OD. Ponseti clubfoot management changing surgical trends in Nigeria,*Lowa Orthop J* 2010;30:7-14.
- [17] Ippolito E, Farsetti P, Caterini R,Tudisco C. Long term comparative results in patients with Congenital Clubfoot treated with two different protocols.*J Bone Joint Surg Am* 2003;85:1286-94.
- [18] Ikeda K. Conservative treatment of Idiopathic Clubfoot. *J Pediatr Orthop* 1992;12: 217-23.
- [19] Dobbs MB, Gurnett CA, Update on Clubfoot: Etiology and treatment. *Clin Orthop Relat Res* 2009 May, 467 (5):1146-1153.
- [20] Majumder ND. Clubfoot. *Neonatal Orthopaedics*.2nd ed. New Delhi: Jaypee Brothers Medical publishers; 2013 p 78-81.
- [21] Boden RA, Nuttall GH, Paton RW, A 14 years longitudinal comparison study of two treatment methods in Clubfoot: Ponseti versus traditional.*Acta Orthop Belg* 2011 Aug;77(4) 522-528.
- [22] Church C, Coplan JA, Poljak D, Thabet AM,Kowtharapa D, Lennan N,et al.A comprehensive outcome comparison of surgical and Ponseti Clubfoot treatments with reference to paediatric norms. *J child Orthop* 2012 Mar;6(1)51-59.
- [23] Turco V J. Resistant congenital club foot . One-stage posteromedial release with internal fixation . *J Bone Joint Surg* 1979; 61-A: 805-814.
- [24] Lehman WB, Ahamed M, Madan ,Sanjeev. A Method for the early evaluation of the Ponseti (Iowa) Technique for the treatment of Idiopathic Clubfoot,*J Pediatr Orthop*.2003;12B(2):133-40.
- [25] Morcuende JA, Dolan LA, Dietz FR, Ponseti IV. Radical reduction in the rate of extensive corrective surgery for Clubfoot using the Ponseti method. *Pediatrics*.2004;113:376-80.
- [26] Abdelgawad AA. Lehman WB. Van Bosse HJ. Scher DM. Sala DA. Treatment Of Idiopathic Clubfoot using the Ponseti method : Minimum 2 years follow-up. *J Pediatr Orthop B* 2007;16:98-105.
- [27] Lourenco AF, Morcuende JA. Correction of neglected Idiopathic Clubfoot by bthe Ponseti method . *J bone Joint Surg Br* 2007;89;378-381.
- [28] Ponseti IV. Eugene NS. Congenital Clubfoot ; The resulta of Treatment. *J Bone Joint Surg*. 1963;45A:2;261-75.
- [29] Chesney D, Barker S, Miedzbrodzka Z, Haites N, Maffuli N. Epidemiology and genetic theories in the etiology of Congenital Talipes equinovarus. *Bull hosp joint dis* 1999; 58:59-64.
- [30] Wynne DR. Genetic and Environmental factors in the etiology of talipes equinovarus. *Clin Orthop*. 1972;84:9-13.
- [31] Boo NY.O.L. Congenital Talipes in Malaysian Neonates: Incidence, pattern and associated factors. *Singapore Med J* 1990;31:539-42.
- [32] Wallander H, Hovelius L and Michaelsson K. Incidence of Congenital Clubfoot in Sweden. *Acta Orthop* 2006; 77(6): 847-52.
- [33] Byron –Scott R, Sharpe P, Hasler C, Cundy P, Hirte C,Chan A, et al. A South Australian Population – based Study of congenital Talipes Equinovarus. *Pediatr Perinat Epidemiol*. 2005; 19:227-37.
- [34] Zosia M . congenital Talipes equinovarus : a disorder of the foot but not hand. *Journ Of Anat*. 2003; 202(1):37-42.
- [35] Tachdjian m. *Pediatrics Orthopaedics*. Philadelphia: Saunders 1972. P 1275.
- [36] Ponseti IV . Clubfoot management. Editorial. *J pediatric orthopaedics* 2000; 20; 699-700.

- [37] Ponseti IV. Relapsing Clubfoot: Causes, Prevention And Treatment. Iowa ORTHOP J 2002;22:55-56.
- [38] Laaveg SJ, Ponseti IV. Long term Results Of Treatment of Congenital Clubfoot. J Bone Joint SURJ Am 1980;62: 23-31.
- [39] Porecha MM, Parmar DS,Chavda HR. Midterm results of Ponseti method for the treatment of congenital Idiopathic Clubfoot (A study of 67 Clubfeet with mean five years follow up) . J Orthop Surg Res 2011;12:3.
- [40] Bor N, Coplan JA, herzenberg JE. Ponseti Treatment for Idiopathic Clubfoot: minimum 5 year follow up. Clin Orthop Relat Res 2009;467:1263-1270.