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High Tibial Osteotomy: Long Term Follow-Up

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KEYWORDS

Arthritis, high tibial osteotomy, long term follow up

ABSTRACT

Pain in the knee joint is one of the most complaints found among the patient attending outdoor. Its existence dates since the adoption of erect posture by mankind. Arthritis may cause mild to moderate pain while to pain at rest joint stiffness varus or valgus deformity. These may withheld a person from his job by preventing him from sitting cross-legged, squat or climb the stairs or walk distances etc. Role of high tibial osteotomy has been studied for prompt relief of symptoms and thus make the patient able to lead a longer independent life. 400 cases were operated during a time period of 11 years from 2005 to 2016. Majority of them were sedentary workers. Majority of the patients have duration of complaints between 1-2 years. The Pain was mostly located on the medial side & the pre operative feeling of instability was absent in majority of cases. The Pre operative range of movement was between 90-120 degrees in 68% of cases. Apart from progressive varus deformity in small gropup of patients and mild pain in the knee joint most patients were benefited by this procedure. 200 cases were operated by wedge osteotomy at the level of tibialtuberosity, 125 below the level and 75 by dome osteotomy. Results were evaluated on the basis of Bailey knee assessment scale. All patients were benefited by surgery as regards the relief of pain. Very old and obese patients showed poor results after surgery due to their inability to do required physiotherapy. Patients having symptoms for shorter duration of showing early evidence of radiological changes responded to surgery better as compared to those having advanced changes. Patient with 1-10° of postoperative valgus in tibio-femoral alignment obtained maximum pain relief and good range of motion. Over correction and failure to achieve valgus showed poor results. 75% of patients showed good results, 20% fair & 5% poor. According to Bailey Knee assessment scale 75% of patients showed good results, 20 % fair & 5% poor. Better resutls were obtained by dome osteotomy as comapred to wedge osteotomy regarding relief of pain, post oerpative range of knee motion, and abilities to walk distances, climb the stairs, squat or sit crosslegged. Osteotomy of fibula in dome osteotomy required special care to avoid complications like pain at fibular osteotomy site and weakness of dorsiflexion of foot. Thus high tibial osteotomy is a boon for early osteoarthritis of knee with unicompartmental involvement and to some extent with moderate to advance osteoarthritis in developing countries as clearly indicated in our study.

Introduction

Arthritis¹ of the knee becomes one of the front liners among the causes of man's disablement. Arthritis may cause mild to moderate pain while to pain at rest, joint stiffness and varus or valgus deformity. These may withheld a person from his job by preventing him from sitting crosslegged, squat or climb the stairs or walk inflammatory distances etc. Anti analgesics drugs, physiotherapy and change in life style comprise essential part of conservative treatment. Various surgical procedures like synovectomy, patelectomy, joint debridement, arthrodesis, arthroscopic surgery and osteotomies and joint replacement have been described. Role of osteotomy have been studied for prompt relief of symptoms and thus make the patient able to lead a longer independent life.

To assess the advantages of high tibial osteotomy by various methods in patients with different grades of osteoarthritis of knee and its long term follow up in those patients.

Material and Methods

The study comprises of 400 patients of osteoarthritis knee which have been operated during the time period of 2005 to 2016 and their follow up has been

evaluated. Apart from progressive varus deformity in small group of patients as well as mild pain in knee joint most of the patients were benefited by this procedure. The procedure was carried out in 3 institutes i.e. 1- S.N. Medical College, Agra, 2- Chandra Orthopedic and Maternity Research Institute & 3-F.H. Medical College & Research Institute.

Each of the patient was clinically evaluated against a prescribed chart, which is as follows –

Investigation

Blood, Urine,

X-ray – A.P. View, in weight bearing, Lateral view and Tangential view.

Weight bearing X-rays were done in all cases to assess severity of varus and valgus deformities. Patients with mild to moderate pain with no evidence of unicompartmental disease were treated conservatively by anti-inflammatory strengthening quadriceps analgesics. exercises. foamentation and intraarticular hydrocortisone injections at weekly intervals.



Fig.-11: Essential instruments and implants : The drill, Staple holder, Osteotome, Staple Impactor and extractor, Curved guide for drill, Plain & Stepped staples and T- handle with guide wire.

High Tibial Osteotomy

Patients revealing diminished joint space in medial or lateral compartment on weight bearing X-ray films were subjected to high tibial osteotomy. Preoperatively amount of valgus or varus deformity was measured by drawing tibial and femoral axes. Closing Wedge osteotomy was done in 240 cases and opening Wedge osteotomy with insertion of graft in 40 cases. Dome osteotomy was done in 120 cases (Dugdale *et al.*, 1992).

Wedge Osteotomy

Wedge osteotomy was done by two methods :

Osteotomy done just at the level of tibial tuberosity (160 cases) (Coventry *et al.*, 1973).

Osteotomy done below the level of tibial tuberosity (80 cases)

Patient anaesthetised and Esmarch's bandage applied to the limb. Lateral longitudinal incision given. Head of fibula osteotomised obliquely. Upper tibio femoral syndesmosis seperated. Wedge of desired size removed with base being laterally for correction of varus deformity 1 mm of base in taken for each 1 degree of correction (Myrnerts Rune, 1980).

Wedge Osteotomy



Size of wedge 'x'mm + 5mm for normal valgus



- Head of the fibula osteotomised obliquely.
- Upper tibiofibular syndesmosis separated.
- Fragments fixed with staples after wedge resection

Size of Wedge : varus in degrees + $5-7^{\circ}$ for normal valgus in mm

Upper level of osteotomy is kept 2 cm away from tibial articular surface which is judged by passing a guide wire through the joint. After removal of the wedge, correction done and 1 or 2



Showing lateral longitudinal incision for wedge osteotomy the knee is held in slight flexion

stepped staples placed in to hold the fragments.

The opening wedge osteotomy is done from medial side and a graft from iliac crest is inserted into the wedge. No staple is applied and cylinder plaster given.



Showing the exposure of head of fibula





Showing the head of fibula being osteotomized obliquely



Showing the tibia being osteotomized from the lateral side about 2cm below the guide wire

Showing the guide wire passed through the joint to assess the plane of tibial articular surface



Showing the wedge resected from the lateral side of tibia



Showing initial insertion of stepped staple after achieving correction

Showing staple being impacted



Showing staples in position

Dome Osteotomy

First fibula Is osteotomised through a small lateral incision in middle third of leg and cut ends of fibula displaced after osteotomy. Another anterior midline incision given and patellar tendon exposed upto tibial tuberosity. A guide wire passed through joint to guide the plane of tibial articular surface. Then a curved guide passed behind the patellar tendon to guide the curve of osteotomy. Multiple drill holds made through this guide into the bone. The guide Is removed and bone is osteotomised Along these drill boles. After the bone is cut in shape of done, distal fragment is rotated into the concavity of proximal fragment till desired correction is achieved. Post operative valgus position is judged by the guide wire into the joint.

OSTEOTOMY

THE DOME



- Curved osteotomy of tibia done above tibial tuberosity
- Fibula osteotomised in mid shaft & fragments displacec
- Distal tibia rotated for correction of deformity.
- Fragments fixed by staples.

Fragments are held In position by 1-2 staples (Sundaram *et al.*, 1986; Benjamin, 1969).

Post operative regimen

Suction drainage for 8 hours was used routinely in every case and cylinder plaster was given. Quadriceps exercises were started within plaster the day of surgery. Partial weight bearing was started after 4 weeks with the help of



Fig.-1: Showing drilling of fibula after exposing it from lateral side in middle third of shaft.

crtuches. Plaster was removed after 8 weeks and check X-rays were taken. Range of motion exercises and full weight bearing was started after evidence of union. Average follow-up period was around 5 years range 2-12 years. Out of 400 operated cases there were 250 females and 150 males. Average age was 55.2 years range 35-70 years.



Fig.-3: Showing guide wire being passed through I the joint to-assess the plane of tibial articular surface.



Fig.-2: Showing the fibula being osteotomised



Fig.-4: Showing exposure of patellar tendon, upto tibial tubrosity



Fig.-5: Showing placement of curved guide for drill to drill the tibia in shape of curve.



Fig.-6: Showing the tibia being drilled through the curved guide.



Fig.-9: Showing final position of staples

Results and Discussion

In our series highest number of cases suffering from osteoarthritis of knee were in sixth decade and lowest between fourth and fifth decades (Table-1). Majority of cases were female (Table-2). Most of the patients were sedentry workers (Table-3) and the duration of complaints was between 1 to 2



Fig.-7: Showing the tibia being osteotomised along the previously dilled holes.



Fig.-8: Showing the stepped staple being insertec into the fragments after correction of deformity years (Table-4). It was seen majority cases pain was very sever and was localized on the medial side of knee (Table-5 & 6). In maximum number of patients instability was absent (Table-7) and history of previous trauma was absent in majority of cases (Table-8). Patients were assessed according to the distance walked, climbing stairs their ability to sit on chair, squating, sitting cross legged and use of aids (Table-9).

It was also noticed that majority of patients have movement of knee between $90-120^{\circ}$ and preoperative flexion deformity was between 0-5° in majority of the cases (Table-10 & 11).

Management of osteoarthritis of the knee joint has ever been a problem. Clinically the symptoms very from mild pain on walking or climbing stairs to moderate or severe pain during day to day activity or even at rest. Also there may develop flexion deformity of varus or valgus deformity at knee.

Pathological changes that take place in an osteoarthritic knee include osteophytes formation along the margins of tibial and femoral condylar articualr surfaces, and the patellar articular surface, degeneration of articular cartilage causing diminution of joint space in medial or lateral or both the compartments of the joint, synovial thickening, degeneration of menisci. presence of loose bodies inside the joint, sclerosis and cyst formation in the subchondral bone. The weakness of vastus lateralis also plays role in making the line of weight bearing to shift medially and thus causing more stress on medial compartment of the knee joint. This further leads to early degenerative changes medial in compartment and varus deformity of knee.

Mode of treatment varies from patient to patient depending upon the underlying pathological changes due to osteoarthritis. management has The evolved from conservative measures in form of foamentation, anti-inflammatory analgesic drugs, excercises. intraarticular hydrocortisone injection (Sarkin, 1972) and procaine needling, measures like to operative Synovectomy 1955). (Steindler, Patellectomy (Coventry, 1987; Ackroya, 1977; Berkeiser, 1939), Joint Debridment (MacIntosh et al., 1977; David, 1977), Femoral and tibial osteotomies about the knee, Arthoscopic surgery and partial and total knee replacement surgery (van Raaij et al., 2009).

400 cases of osteoarthritis knee with unicompartmental involvement were operated during a time period of 12 years (2004-2016) and results were evaluated. The procedure undertaken was 1. wedge osteotomy at the level of tibail tuberosity, 2. wedge osteotomy below the level of tibail

tuberosity and 3. dome osteotomy. It was observed cases above 65 years of age could obtain only fair results in the series Majority of the cases with good results were below 65 years of age. The possible reason was inability to do physiotherapy. Obese pateints were found to wrong selection for operation. In our series local of more severe pain was found to be medial (71.6%) cases, 16.6% anterior, 6.6% lateral and 5% had posterior. This clinical observation along radiological examination leads to conclusion that medial compartment is first and most common to be involved. Next commonly there is involvement of patellofemoral joint (Insall et al., 1984; Benjamin, 1969; Jakob et al., 2004).

The duration of preoperative complaints has greatly influenced the outcome of surgery. The best results were obtained in patients with a history of illness for less than one year. In this series 69.2% of cases having complaints for less than 1 year showed good results while only 44.1% showed good results with duration of complaints for over one year.

It may be argued that some of the patients with lesser duration of preoperative complaints could have done well, anyhow, if thev were treated with conservative measures. This could well be so, but does not take into account the long period of pain and disabilities and the loss of time from work that would have been involved. It may patients be recommended that not responding to conservative measures within 6-8 months or having relapsed within a year should be subjected to surgery.

Improvement in pain was observed in all patients and no case had severe pain persisting postoperatively. Preoperative moderate to severe pain was present in 91.6% cases while there were only 5% cases with mild pain after operation.

Function was also greatly improved in patients after operation. All patients could walk after surgery. 50% of the patients could do outdoor activities as compared to 10% preoperatively. Only 12 cases were using stick for walking postoperatively while 100 were using it earlier. All patients were able to sit comfortably in chair without any difficulty while 18.3% had difficulty previously.

Overall there were 61.6% cases who could climb the stairs preoperatively. Postoperative improvement occurred in 91.6% cases. Preoperatively 26.6% of patients were unable to sit cross legged. They reduced to 13.3% after operation. One third of total cases could sit cross legged after operation as perfectly normally preoperatively. compared to 18.3% Squatting abilities of patients also improved. Over 80% could squat after operation compared to 36.6% preoperatively.

Instability or insecurity while walking was complained by 18.3% cases preoperatively reduced to 13.3% cases postoperatively. It was found that most of the patients (66.6%) were able to sit on chair (flex upto 90°) within one month and a few (13.3%) could flex upto 120°. After 6 months of removal of plaster 86.6% of cases could flex beyond 90% out of them. 26.6% had flexion beyond 120° and 60% between 90-120°.

The results of high tibial osteotomy were very encouraging. In our series at osteotomy, attempt was made to correct flexion and varus or valgus deformities at knee due to osteoarthritis. Previously 21 cases (35%) had flexion deformity at knee, which was corrected in majority of cases.

Tibiofemoral alignment which required correction, was varus in 50%, 0° in 50 cases and more than 10° valgus in 10 cases. At surgery 5-7° of valgus was aimed.

No attempt at over correction was made. Postoperatively 60% had valgus of 1-5° and 28.3% between 6-10°. 5% cases had valgus more than 10° (Table-12).

Age group (yrs)	No. of cases operated	Percentage
18–30	-	-
31–40	13	3.3
41–50	14	3.3
51–60	313	78.3
Above 60	60	15

Table.1 Showing the age distribution

Sex	No. of cases operated	Percentage
Male	150	37.5
Female	250	55.5

Table.2

Table.3 Showing distribution of quality of life

Quality of life	No. of cases operated	Percentage
Heavy manual workers	75	18.3
Sedentary workers	166	41.7
Retired life	113	28.3
Unable to do any work	46	11.6

Table.4 Showing duration of complaints

Duration	No. of cases operated	Percentage
Less than 6 months	61	15.0
6 months – 1 year	113	28.3
1 year -2 years	153	38.3
More than 2 years	73	18.3

Table.5 Showing severity of preoperative pain

Severity of Pre-op. pain	No. of cases operated	Percentage
Severe	226	56.6
Moderate	140	35.3
Mild	34	8.3
None	0	_

Table.6 Showing location of most severe pain

Location	No. of cases operated	Percentage
Medial	286	71.6
Lateral	26	6.6
Anterior	68	16.6
Posterior	20	5.0

Table.7 Showing preoperative feeling of instability/insecurity while walking

Insatability/ Insecurity	No. of cases operated	Percentage
Present	73	18.3
Absent	327	81.7

Table.8 Showing history of previous trauma

History of previous trauma	No. of cases operated	Percentage
Present	26	6.6
Absent	374	93.3

Table.9 Showing preoperative abilities of patient

Preoperative abilities	Percentage
Distance walked	
• Unlimited	12
• Limited	35
• Indoors only	48
• Unable	5
Climbing stairs	
• Easily	20
• With difficulty	62
• Unable	18
Sitting in Chair	
• Easily	92
• With difficulty	8
• Unable	-
Squating	
• Easily	7
• With difficulty	30
• Unable	63
Sitting Cross legged	
• Easily	19
• With difficulty	55
• Unable	26
Use of Aids	
No aids	70
• Stick	30
Crutches	-

	No. of cases operated	Percentage
0-60°	-	-
60-90°	26	6.6
90-120°	273	68.3
More than 120°	101	25.0

Table.10 Range of preoperative movement

Table.11 Preoperative flexion deformity

Flexion deformity	No. of cases operated	Percentage
0-5°	132	20
6-10°	80	11.6
11-15°	46	3.3
More than 15°	-	-

Table.12 Showing tibifemoral alignment on standing

Tibiofemoral alignment	No. of cases operated	Percentage
0°	66	16.6
Valgus		
• 1-5°	120	30
• 6-10°	0	-
• >10°	13	3.3
Varus		
• 1-5°	93	23.3
• 6-10°	60	15.0
• >10°	46	11.6

Table.13 Numebr of cases

HTO at the level of	HTO below the level of	Dome
tibial tuberosity	tibial tuberosity	Osteotomy
200	125	75

Assessment of results was done on the basis of following criteria. At every follow up points scored by the patient were noted and results were graded accordingly (Table-14).

Criteria	Points scored	Total
Pain		
Severe	0	15
Moderate	6	
Mild	12	
None	15	
Function		
Walking distances	5	20
Walking aids	5	
Stair climbing	3	
Resting in chair	2	
Giving way	5	
Movements		
1 point for each 12° (Max. 120°)		10
Deformity		
No fixed flexion or lag	3	5
Varus/valgus angle	2	
Total		50

Table.14 Showing criteria on which results were assessed and graded bailey knee assessment scale

GRADING OF RESUTLS GOOD

FAIR

POOR

POINTS SCORED 35-40

30-34

<30

Table.15 SHOWING LENGTH OF FOLLOW UP

Period (years)	No. of cases operated	Percentage
< 1 year	72	18.0
1-2	120	30.0
2-4	64	16.0
4-6	72	18.0
6-8	40	10.0
8-10	32	8.0

Parameters (postoperative)	HTO at the leve	l of TT	HTO below the lev	el of TT	Dome osteot	tomy	P value
	No. of cases	%	No. of cases	%	No. of cases	%	
Pain							
• None	76	38.4	40	32.0	32	43.7	>0.8
• Mild	84	42.3	50	40.0	32	43.7	>0.3
Moderate	38	19.2	32	26.0	9	12.5	0
• Severe	-	-	-	-		-	0
Range of motion							
• 120°	69	34.6	40	32.2	32	43.7	>0.9
• 90-120°	115	57.6	68	54.6	42	56.3	-
• 60-90°	15	7.8	25	20.0	28	-	-
• <60°	-	-	-	-	-	-	-
Function							
Distance walked							
• Unlimited	61	30.7	33	26.8	33	37.5	>0.01
• Limited	107	53.9	63	50.8	37	50.0	>0.01
• Indoors only	30	15.3	26	20.8	9	12.5	-
• Unable	-	-	-	-	-	-	-
Climbing stairs							
• Easily	153	76.9	90	72.0	60	81.3	< 0.1
• With difficulty	38	19.2	30	24.0	14	18.7	>0.5
• Unable	8	3.8	10	8.2	-	-	< 0.02
Squating							
• Easily	107	53.9	58	46.8	37	50.0	< 0.01
• With difficulty	69	34.6	47	38.0	32	43.7	>0.10
• Unable	22	11.4	19	15.2	4	6.3	< 0.05
Sitting cross legged							
• Easily	115	57.6	62	50.0	37	50.0	< 0.10
With difficulty	69	34.6	47	38.0	37	50.0	< 0.50
• Unable	15	7.8	15	12.0	-	-	< 0.02

Table.16 Showing comparative results of HTO at the level of TT, HTO below the level of TT and dome osteotomy

Thus in our table painlees kneen after dome osteotomy was present in 43.7% as compared to 38.4% in cases of wedge osteotomy (Table-16).

Table.17 Showing incidence of complications

Complications	No. of operated cases	Percentage
Superficial infection	20	5.0
Deep infection	-	-
Non-union of osteotomy	-	-
Weakness of dorsiflexion of foot	12	3.0
Pain at site of fibular osteotomy	33	8.3
Fracture into the joint	8	2.0



Preop. X-ray showing diminished joint space in medial compartment and varus 7^o on left side



Immediate post-op. X-ray after wedge osteotomy tibio femoral alignment has been made valgus 5°







Showing functional Results



Check X-ray after 1 year follow up the valgus is maintained



CASE NO.2



Pre-op. X-ray showing bilateral varus deformity at knee and diminished joint space on medial side. Operated side L had varus of 7°



Post-op. X-ray after dome osteotomy space in medial compartment has appeared the fibula is osteotomised in mid. third

CASE NO.2



Post-op. at 3 months. Valgus is maintained. healing is in progress



Post-op. at 9 months. Correction is maintained. osteotomy has healed











CASE NO. 3



Pre-op. X-ray showing involvement of lateral compartment with 15° Valgus and flexion deformity at knee



Pre-op. valous deformity and amount of knee flexion

CASE NO. 3



Immediate post-op. showing wedge resection from medial side and space in the lateral compartment is created. Valgus is reduced to 6°



14 months follow up with maintenance of correction and healing of osteotomy





Showing post-op. corrected deformity of the limb and functional result. Post-op full flexion at knee enabled him to do his job satisfactorily

CASE NO.4



Pre-op. X-ray showing varus deformity and diminished joint space on medial side in left knee (7°)



Immediate post -op. showing intraarticular fracture of lateral condial of tibia. This occurred while attempting correction in wedge osteotomy



Check X -ray after 3 months showing evidence of healing. The corrected tibiofemoral alignment to valgus is



18 months follow -up fractured healed and valgus







Showing functional resutls

CASE-5



Pre.-op.



Immediate Post-op.



3 months follow-up Valgus is maintained

CASE-5



3 months follow-up

3 months follow-up

CASE-5



1 year follow-up

1 year follow-up

CASE-5



3 years follow-up

5 years follow-up

CASE-6



Pre-op. X-ray



10 years follow up



10 years follow up



20 years follow up



20 years follow up



Showing the present status of the patient

Another signification observation regarding the relationship between postoperative tibiofemoral alignment and persistent pain was made. Cases with 1-10° of valgus alignment (71.5%) cases had none or mild pain only. All cases with overcorrection (6.6%) had persisteing moderaly severe pain. This finding is same as observed by Hiromu S. and John Insall (1977) but there are many others who favoured are

recommended overcorrection eg. P.V. Seal and R.N.W. Chan (1975) Donald B. Kettelkamp Wenger and Corolyn Thompson (1976) J.P. Blanched, Glord, JH Merotte, JL Gullams and J.P. Besse (1979) and Anthony F. Pachelli and Engene E. Kaufman (1987). There is third group of surgeons represented by Appel H. and Friberg S (1972) who believe that "Osteotomy had a very good and long lasting effect upon pain at rest, regardless of postoperative varus or valgus angle and the effect of pain in motion is less predictable".

In present sereis, results of wedge and dome osteotomies were compared. It was found that dome osteotomy gives better results as comapred to wedge osteotomy and wedge osteotomy at the level of tibial tuberosity give better results than wedge osteotomy below the level of tibial tuberosity (Sarkin, 1972).

Painless knees following dome osteotomy resulted in 43.7% cases as comapred to 38.4% cases of wedge osteotomy. Knee flexion of more than 120° was obtained in 43.7% cases of dome osteotomy as comaprd to 34.6% cases of wedge osteotomy. 37.5% cases of dome osteotomy could walk out doors for unlimited distances as comapred to 30.7% cases of wedge osteotomy.

43.7% cases of dome osteotomy could squat easily compared to 34.6% cases of wedge osteotomy. Similarly 50% cases of dome osteotomy were able to sit crosslegged as comapred to 346% cases of wedge osteotomy.

The above comparison proves that dome osteotomy gave better functional results as comapred wedge osteotomy in this series. It is important to note there was weakness of dorsiflexion of foot and pain at site of fibular osteotomy which was due to fibular osteotomy in mid shaft. It seems that few nerve fibres to extensor Hallucis and Ext. dig. Longus are injured during fibrular osteotomy and they are responsible for weakness of dorsiflexion of foot or the great toe only (Table-17).

Intraarituclar fracture was noted in 8 cases of wedge osteotomy all these had preoperative genu varum deformity. While osteotomising through the upper tibia and removing the wedge, medial cortex was undevided and correction was attempted in this position which led to the fracture of tibial condyle. In these cases plaster immobilization was done till there was radiological evidence of union.

Conclusion

In our study it was seen that all patients were benefited by surgery as regards the relief of pain. Very old and obese patients showed poor results after surgery due to their inability to do required physiotherapy. Patients having symptoms for shorter duration and showing early evidence of radiological changes responded to surgery better as compared to those having advanced changes. Patient with 1-10 of postoperative valgus in tibio-femoral alignment obtained maximum pain relief and good range of motion. Over correction and failure to achieve valgus showed poor results. 75% of patients showed good results, 20 % fair & 5% poor. Better results were obtained by dome osteotomy as compared to wedge osteotomy regarding relief of pain, post oerpative range of knee motion, and abilities to walk distances, climb the stairs, squat or sit crosslegged. Osteotomy of fibula in dome osteotomy required special care to avoid complications like pain at fibular osteotomy site and weakness of dorsiflexion of foot. Thus high tibial osteotomy is a boon for of with early osteoarthritis knee

unicompartmental involvement and to some extent with moderate to advance osteoarthritis in developing countries as clearly indicated in our study.

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