Comparison Of Formalin Preserved Bone Allograft In The Form Of A Paste And As Bone Chips In Fresh Femoral Shaft Fractures With Comminution

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ABSTRACT

Femoral shaft fractures with comminution are indicators for primary bone grafting. Problems related to autograft use have led us to evolve methods using allografts preserved in formalin. This method is cheap, easy, reliable and is readily available in the poorer countries. Comparison of 20 cases using allograft paste with 20 cases using allograft chips slivers in similar femoral shaft fractures was done. In both cases, the fusion time was slightly delayed as compared to cases using autograft. The infection rates were comparable, but it was noticed that bone paste got resorbed in 2 cases, leading to plate bending and malunion. The theoretical advantage of the bone paste in filling up the defects and crevices in the comminuted fractures was offset by the practical disadvantage of having no osteoconductive scaffolding, which was provided by the bone chips. It is thus recommended that formalin preserved bone allograft paste may be better suited for use in bony cavities and joint replacements, while formalin preserved bone chips are a good alternative to bone autografts, especially in polytrauma cases. Formalin preserved allografts are a viable alternative method for use in third world countries.

Keywords: bone graft, formalin preserved femur fractures

INTRODUCTION

The use of cancellous bone grafting in high velocity fractures with comminution reduces the chances of delay in union and implant failure. The problems related with use of autograft are additional morbidity and local complications, especially in patients of polytrauma. Allografts therefore, have become a viable alternative; routine allograft use, however, is restricted by the high cost, sophisticated storage equipment and limited availability. Keeping these factors in mind, we have developed protocols of allograft use and storage which are simple and cheap, making these ideal for the under-developed countries. Good results have been seen in experimental land clinical settings using 0.5% formalin as a medium of allograft storage[1-3].

Pulverisation of bone to powder form and subsequent decalcification have been shown to enhance the osteoinduction potential of the graft, perhaps due to bone morphogenic protein (BMP) and fibronectin[4-7]. Bone in the form of powder is thought to allow a greater exposure of the particles of the osteoinductive substance to the host cells due to larger surface area[4]. The small size of the particles may also allow easier neovascularisation as compared with larger bone chip or inlay grafts[4].

Bone graft, used in the form of a paste, has the advantage of easy application and placement, especially in larger skeletal defects or cavities and comminuted fractures. The bone paste is easy to prepare from the processed allograft slivers using a powered bone mill. At our institute, demineralised bone matrix as a paste was used in large segmental defects in the ulna of rabbits, with excellent results and minimal complications[3]. Combining two principles, this prospective clinical study was conducted to evaluate the role of formalin preserved allograft in the form of paste in fresh fractures with comminution. The principle employed was that the paste would fill up the defects in the bone created by the comminution and fracture union may be improved. These results were compared with a previously published series of similar cases where formalin preserved allografts were used as chips/ slivers in similar cases.

MATERIALS AND METHODS

Twenty consecutive cases presenting with closed comminuted fractures of the femoral shaft were selected for the study during the period 1992 to 1994. The criteria for selection of the graft recipients were comminuted femoral diaphyseal fractures (grades according to Winquist and Hansen), which were not compound, had no systemic injury and could be operated within 3 weeks of injury. Informed patient consent was obtained in all cases. All these cases were followed-up till fracture union or failure of union, and the time to union was assessed. Four weekly serial radiographs were taken to assess the progress of the fracture and correlated with clinical features.

Standard techniques of AO fixation were used. The graft paste was used to fill up the comminution gap and also implanted all around the fracture site so

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Table I - Parameters for evaluation of results

GOOD	Complete bridging of fracture line with or without trabecular pattern restoration at 8 months post-operative.
FAIR	Malunion or incomplete bridging of fracture line at 8 months post-operative.
POOR	No signs of bridging a fracture line at 8 months post-operative. Implant failure. Evidence of deep infection.

Table II - Details of 20 cases

S. No.	Fracture type	No.	Comminution* grade			Implant used		Additional circlage wire
			II	II	III	DCP	ABP	
1.	Proximal third	2	-	1	1	2	-	-
2.	Middle third	12	3	8	1	12	-	-
3.	Distal third	6	1	3	2	3	3	1
	Total	20	4	12	4	17	3	1

* According to Winquist RA and Hansen ST: Intramedullary nailing of femoral shaft fractures. Orthop Clin North Am 1980; 11:633-48.

Associated fractures were seen in 14 cases.

as to form a strut, and the comminuted fragments were replaced over it. Anatomical reconstruction of the bone was attempted with minimal devascularisation of the fracture fragments. An attempt was made to fix the fragments with interfragmentary screws and circlage wires. In cases with fractures of both the limbs, the graft paste was used for one fracture alone (except in one case where the opposite fractured femur was fixed with an intramedullary nail and derotation plate, and supplemented with allograft bone paste).

The blood groups of the donor and the recipients were matched.

All patients were given broad spectrum intravenous antibiotics for 5 days and then oral antibiotics till suture removal.

Graft source, procurement and storage

Bone graft was obtained from cancellous/ metaphyseal sites from young healthy donors undergoing amputation for traumatic conditions and femoral heads removed at time of total hip arthroplasty. All aseptic precautions were used and these patients were repeatedly examined for any evidence of infectious diseases (including hepatitis & HIV). The bone graft was cleared of soft tissues, treated for 15 minutes with absolute alcohol, and washed with solvent ether for 15 minutes. These bone pieces were stored in 0.5% formalin solution in 0.9% saline at pH 6.2-6.4 and kept in ordinary refrigerators at 2-4°C. The graft was rinsed weekly with normal saline, using all aseptic precautions, and the formalin solution was changed for a minimum period of 3 weeks, before it was ready for use. Repeated samples were sent for culture. At the time of use, the graft was rinsed with sterile saline and transferred to a bowl containing 1 gm cloxacillin and 80 mg gentamicin for 30 minutes. The graft was then dried with a sterile sponge and pulverised with a powered bone mill. The powder was mixed with distilled water to form a paste prior to use.

Follow-up assessment

All the cases were reviewed at monthly intervals. Those with sterile non-union were reoperated using autogenous cancellous graft with or without change of implant. Serial X-rays were done to check for fracture union and graft incorporation. The results were graded according to Table I.

OBSERVATIONS AND RESULTS

There were 18 males and 2 females. The age of the patients ranged from 17 years to 46 years (average 30 years). No case had any systemic injury, but 12 cases had associated fractures of other bones, with 4 patients having bilateral femur fracture. Follow-up ranged from 8 months to 16 months (average 10 months).

Seventeen cases were fixed with broad DCP and 3 with 95° angle blade plate with or without interfragmentary screws and circlage wires. Two cases of bilateral femur fracture were fixed with intramedullary nails and 2 with AO plates on the opposite side. The details of the cases are given in Table II.

Fig 1a - Preoperative radiograph of comminuted fracture shaft of femur.

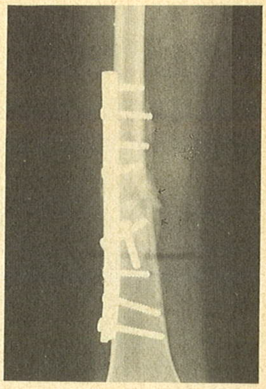

Fig 1b - Post-operative radiograph following open reduction and internal fixation along with the application of allograft bone paste.

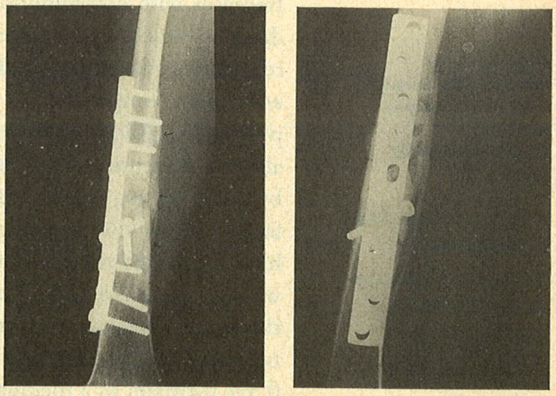

Fig 1c & 1d - Post-operative radiograph of the same case at six months follow-up (AP and lateral views). Complete bridging of the fracture site with incorporation of allograft is seen.

Fig 2a - Pre-operative radiograph of comminuted fracture of shaft of femur.

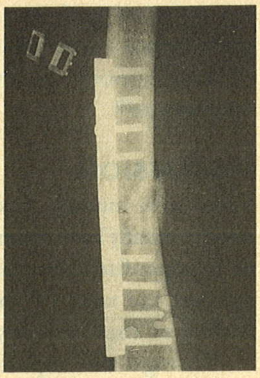

Fig 2b - Immediate post-operative radiograph of the same case. The allograft bone paste is seen placed at the fracture site.

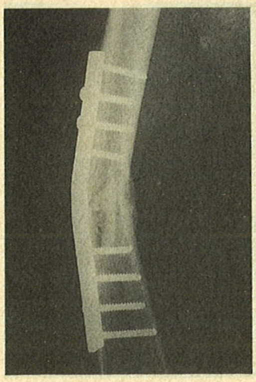

Fig 2c - Same case at six months follow-up. The fracture has united in a varus position with the plate bent. The allograft is seen buttressing the medial cortex and is fully incorporated with the host bone.

Fig 3a - Pre-operative radiograph of fracture shaft of femur with Gr.III comminution.

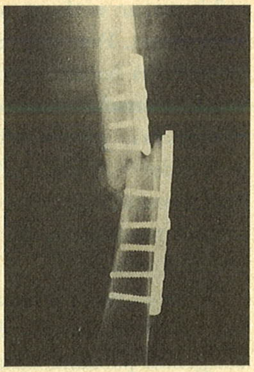

Fig 3b - Post-operative radiograph of the same case at six months follow up with plate breakage.

Fig 3c - Post-operative radiograph of the same case after removal of the plate and revision of fixation using an intramedullary nail and supplemented with derotation plate and autogenous cancellous graft.

None of the cases developed inflammatory reaction either locally or systemically in the immediate post-operative period. Blood group matching was not a significant factor since good union rates were achieved even in unmatched cases.

Sixteen fractures united (13 good, 3 fair) and there were 4 poor results (Table I). The average time for fracture union was 6.5 months (range 5-8 months). One case had a delayed union at 10 months post-operatively.

All the 13 cases with good results (Fig 1) and the 3 with fair results showed complete bridging of fracture line as seen on X-rays. All these cases were weight bearing fully at average 7 months follow-up (range 6 to 8 months).

Complications

In 2 cases labelled as malunions, the plate got bent when the patient started bearing weight before he was advised to do so. The fracture united in a varus position (Fig 2). However, both the patients were able to bear full weight by the end of 6 months and refused any surgical intervention. In another case, the fracture did not unite by 8 months and radiographs revealed incomplete bridging of fracture site. He was put on a cast brace and the fracture subsequently united 2 months later. He was labelled as delayed union.

Four cases went into sterile non-union. In one case, the plate broke at the fracture site at 6 months post-operation. The plate was removed and the fracture revised using an intramedullary nail supplemented with derotation plate and cancellous autograft. The allograft was found incorporated with the host bone above and below the fracture site. Fibrous tissue was interposed at the fracture site and the medullary canal was closed. However, the fracture ends were not sclerosed. A biopsy taken from the host bone allograft interface (Fig 3) revealed ingrowth of lamellar bone into the allograft along with scattered multinucleated giant cells. Another case presented with a sterile gap non-union at the fracture site at 6 months. Cancellous autograft was placed at the fracture site and the fracture united uneventfully. The fracture site and the fixation were not disturbed. In the third case, the plate got pulled off from the distal fragment at 4 weeks post-operation when the patient lost balance and fell down. The patient was reoperated and the fixation was revised using an intramedullary nail. The bone paste was left in situ. After protected weight bearing for 3 months, the fracture united with 2 cm shortening. The fourth patient, a case of bilateral fracture shaft of femur where plate fixation and allografting were carried out on both sides, presented with varus deformity and pain at fracture site on the right side at 3 months follow-up. The patient had put weight on the limb against advice. The fracture showed no signs of union on serial radiographs. He was reoperated and cancellous autograft was put on the medial surface opposite the plate. The fracture united in varus 2 months after surgery. Only 2 cases in this series had restricted joint motion at the knee of less than 90°.

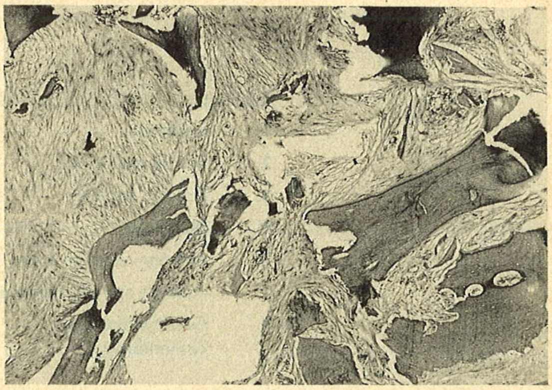

Fig 3d - Micro photograph (x40) of the biopsy taken from fracture site at the host bone allograft interface during the revision surgery. In-growth of lamellar host bone into the allograft is seen.

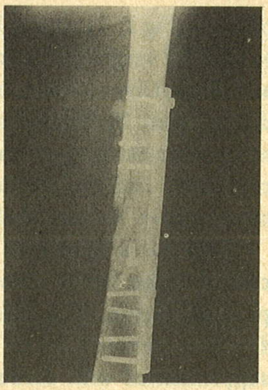

Fig 4a - Immediate post-operative of photograph following internal fixation of comminuted fracture femur along with application of allograft bone paste. The allograft is seen as a mass of bone with indistinct margins. The bone paste has a lower density as compared to host bone.

Fig 4b - Post-operative radiograph at the end of six weeks. An increase in the density of bone paste is noticed and is seen bridging the fracture site.

Radiological findings

In the immediate post-operative period the bone paste was seen as a mass of bone with indistinct margins (Fig 4a). The paste had a lower radiodensity as compared to the adjacent fracture fragments. The radiodensity of the paste reduced further by the end of 4 to 6 weeks. At 6 to 8 weeks, an increase in the density of the bone paste was noticed and it was seen bridging the fracture site (Fig 4b). The density progressed to reach its maximum at 5 months at which the allograft was incorporated with the host bone. In 2 cases, the bone paste was found to be resorbed and was not visible on radiographs by the end of 8 weeks. In another series of 22 comminuted shaft fractures, where formalinised allograft in the form of cancellous bone chips was used to supplement plate fixation, no resorption of graft was noticed in any case and the graft formed a scaffold over the compressile surface of bone opposite to the plate, thus bridging the fracture site[4].

DISCUSSION

Cases with multiple fractures due to high velocity trauma usually have extensively comminuted fractures with jeopardised vascularity; numerous authors have stressed the need of cancellous bone grafting for healing of these severely comminuted fractures[8-12].

Though the superiority of fresh cancellous autograft over allograft is well established, there are often limitations to its use in the form of limited quantity, shape, size and patient morbidity. Cancellous allograft provides a good alternative especially in cases with polytrauma, where minimal surgery is desirable.

Formalin as a preservative has been shown to have distinct advantages over other methods[13-15]. It is cheap, easy to prepare and can be stored in an ordinary refrigerator without any need for sophisticated equipment. Previous authors have observed that formalin is bacteriostatic, and the tissues stored in weak formalin solution retain their biological, structural, physicochemical, and mechanical properties[13]. Additionally, weak solutions of formalin may alter and reduce the antigenic properties of water soluble proteins in the tissues, resulting in reduced immune reaction[15].

Various reports are available about the successful use of formalinised bone graft for nonunions, congenital pseudoarthrosis, for packing cystic defects, in achieving spinal fusion and in protrusio acetabuli[16,17]. This method is developing into a good alternative to sophisticated and expensive techniques in the under-developed countries with limited resources. A previous study has shown good results with allograft bone chips stored in formalin[2]. The study was undertaken with the aim to see if bone allograft paste had any significant advantages (in filling

Table III - Comparison of results

S.No	Complication	Ruedi & Luscher Study (1978) autograft	Vancouver study (1980) autograft (14/46 cases grafted)	Daminot et al., autograft (1989)	Mathur et al., allograft chips (1994)	Present study allograft bone paste (1995)
1.	Infection	6%	7%	7%	5%	0%
2.	Sterile non-union	7%	2%	7%	10%	5%
3.	Implant (failure)	7%	13%	3%	5%	15%
4.	Malunion	0%	3%	6%	-	10%
5.	Average time for union	5 months	7 months	5 months	6 months	6.5 months
6.	Average age of patient (yrs)	40.8	18	28	34	30
7.	Grade of fracture comminution	Gr. not specified	Gr. not specified	Gr. I 18 Gr. II 11 Gr. III 9 Gr. IV 3	Gr. II 10 Gr. IV 10	Gr. II 4 Gr. III 12 Gr. IV 4

ATG = autograft
ALG = allograft
Gr. = Grade of comminution

up the crevices of comminuted fractures) over the routinely used formalin preserved allograft bone slivers.

On the negative aspect, bone paste lacks the mechanical properties of strip grafts and is more liable to get displaced by muscular movements. It also requires prolonged fixation till new bone formation and consolidation of the graft. It is non-viable and uncontrolled in growth and the smaller particle size increases the chances of it getting resorbed[3,18]. On comparison of our cases done using bone paste and those done using bone chips, a few significant differences were noted. In 2 cases, the bone paste was seen to get resorbed, which was never a problem in the cases where bone chips were used. The bone chips always formed a scaffolding on the side opposite to the plate within a few weeks; this was an important factor in 2 of our cases grafted with bone paste who developed malunion. Less than optimal fixation with the AO plate had left a cortical defect in the bone and as the paste did not enhance any early mechanical stability, the plate bent till the bone ends came in contact. This resulted in malunion, which may reflect upon the better stability potential of the bone chip grafts, which may provide a scaffolding for early bone in-growth in addition to bone induction, if any.

In our study we have observed 80% union rates. This compares favourably with previously reported studies using auto and allograft (Table III). A notable feature in this comparative evaluation is that in all the above-mentioned studies, plating with autografting was done in all grades of femoral fractures, whereas in our study only cases with comminuted fractures were included.

In our study, the average time for union has been 6.5 months whereas in cases of fracture fixation with autografts fractures have united at an average of 4-5 months. In a study using formalinised allograft slivers, the average time for union was 6 months. The longer time taken for union of fractures using formalinised allograft in paste form could be due to the failure of the paste to provide the initial scaffold for ingrowth of new bone. Inadequacy and displacement of the graft paste from the fracture site and non-viability of the allograft in general may also be a factor for increased duration taken for fracture healing in our study.

Since it has been proven beyond doubt that bone grafting in some form is essential for early healing of comminuted fractures, the present study only attempts to compare the effects of formalinised allografts in the form of chips and paste, upon the times to fracture union. Although this is a small series, certain facts are essentially clear. The importance in understanding the big-dynamic effects of fracture repair under rigidly fixed conditions is that the fracture has to be protected at the critical time when the resorptive phase has outstripped the appositional phase. Although it is not clear at what exact times these phases occur in humans, it is amply clear that in addition to age, physiological repair capacity, size and fracture location, autografts lead to faster healing times than allografts[19]. This is probably due to the bone induction capacity which autografts possess as compared to allografts. The comparison between 2 types of formalin allografts used is based clearly on the bone conduction ability of bone slivers/ chips being better than bone allograft paste. The paste was used to fill up the crevices and cracks of the comminuted fractures, but it did not lead to faster healing times. In fact, in cases with less than optimal AO fixation methods, the fracture tended to collapse till the cortices opposite to the plate came in contact, leading to malunion. This problem was not encountered in cases with bone chip allograft, where the slivers acted as scaffolding for bone conduction opposite to the plate, and there was early load sharing between the new bone and the plate. The theoretical advantage of the bone paste filling up the crevices of comminuted fractures was significantly offset by this problem. In none of our cases, however, using formalin as a preservative medium for allografts, was there any evidence of graft rejection, any immune reaction in the form of inflammatory changes or regional lymphadenopathy. The cases where the fixation failed were probably due to faulty fixation techniques; only in one case of sterile non-union was the graft completely resorbed/displaced, requiring autografting, without any fracture fixation changes.

In conclusion, formalin preserved allografting is now an established method at our center, with bone chips being the allograft of choice for comminuted fractures. Formalinised allograft paste should be reserved preferably for bony cavities or for joint replacements, although a combination of both may occasionally be desirable. When using allografts, however, longer times to fracture union should be envisaged and weight bearing has to be appropriately deferred. The methods however are excellent for multiple injured patients, especially when more than one fracture has to be grafted.

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