Pasteurized osteo-articular autologous graft for the reconstruction of proximal humerus after resection of osteosarcoma

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Objectives
For skeletal reconstruction in surgery for bone tumors, pasteurization of the bone has been used with favorable results over other methods of recycling.
Patients and methods
Ten patients with osteosarcoma of the proximal humerus were treated by wide margin resection and reconstruction with pasteurized osteo-articular autologous bone graft. They were seven females and three males, between 9 and 30 years of age who were followed up for at least 3 years (mean, 48 months). The International Society of Limb Salvage graft evaluation method was used for the evaluation of the radiographs.
Results
Six (60%) patients had complete incorporation of the graft and three (30%) patients had partial incorporation. The mean radiographic scoring rate was 84%. Viability of the grafts was evaluated by bone scintigraphy. Of the nine patients evaluated, uptake was detected in seven patients from ~6 months postoperatively after which it increased gradually. The functional results were assessed by the system of the Musculoskeletal Tumor Society and the mean functional rating was 90%. Seven patients have been disease free and three have died of the disease. Resorption of the graft was seen in a single, 9-year-old patient (10%); no fractures or infections were seen. No local recurrence was detected.
Conclusion
These results indicate that pasteurization of the bone may be a useful option for reconstruction after resection of osteosarcoma of the proximal humerus. The advantages of extracorporeal pasteurization include convenience of use, avoidance of intraspecies infection and allogenic reactions, and satisfactory bone remodeling.

Keywords:
Osteosarcoma, pasteurized osteo-articular autologous graft, reconstruction

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Introduction

Limb salvage surgery for treatment of malignant bone tumors is an acceptable alternative to amputation in most patients as marked improvement has been achieved in diagnostic imaging, neoadjuvant chemotherapy, and operative techniques [1–5]. The survival rate of patients with high-grade bone sarcomas has improved from less than 20% [5–7] to ~80% with modern protocols of multidisciplinary treatment. Established reconstructive techniques have well-documented high rates of specific problems and complications [2,4,8–14]. Fracture incidences between 11 and 45% have been reported for allografts and infection rates vary between 7 and 30% for limb salvage surgery [2,4,12,15]. Allografting is a commonly used procedure for reconstruction; however, with the use of an allograft there are concerns of immunologic reaction, transmission of diseases, and religious and social dissatisfaction in some regions of the world. Therefore alternative techniques are worth considering. Immediate reimplantation of the resected bone in the surgical treatment of cancer can be done after extracorporeal treatment such as heating [1,6,7,10,11,16], freezing [17], or irradiation [3,18], to devitalize the tumor-infiltrated segment. Heating is simpler than other procedures in devitalizing tumor cells. Studies and clinical applications of the hyperthermia-treated bone proved the superiority of pasteurization of bone, which is a treatment of heat at 60–65°C for 60 min, over other methods of heat treatment such as autoclaving or boiling possibly attributable to loss of osteogenic potential resulting from high temperatures [1,6,7,10,11,16,19–21]. The superiority depends on the lethal effect of pasteurization on malignant cells while preserving the bone-inducing property [1,6,7,10,11,16,19–22]. Urist *et al.* [23] showed that bone induction is produced by

the response of the mesenchymal cells in the recipient bed to bone morphogenetic protein (BMP) transferred from the bone implant. Urist and colleagues also showed that in thermal exposures greater than 70°C, or irradiation sterilization, the biologic activity of BMP is completely destroyed. The recent concept of hyperthermic oncology suggests its applicability to thermal treatment of the resected bone for immediate reimplantation [1,6,7,10,11,16,19–22,24,25]. The current study examined the influence of extracorporeal hyperthermia using the pasteurization method on reintegration of autologous osteochondral grafts to determine its validity for reconstruction after resection of malignant tumors of the proximal humerus. The tumor is resected with wide margins [26], and macroscopic tumor then is cleared before treatment with hyperthermia. Reconstruction is done by immediate replantation of the devitalized graft and stabilization by suitable osteosynthesis [1,6,11,22]. Osteochondral grafts were used in all patients. Although cartilage degradation is inevitable, the functional deficits would be less as the shoulder is a non-weight-bearing joint.

This report describes the clinical results of 10 patients with osteosarcoma of the proximal humerus. All had intra-articular resection. For the assessment of reintegration of the grafts, the radiographic evaluation method of the International Society of Limb Salvage [15] was adopted. Viability of the grafts was evaluated by bone scintigram according to the method of Ehara *et al.* [7,16], and oncologic and functional results were assessed using the Musculoskeletal Tumor Society system [9].

Patients and methods

Between April 2003 and January 2008, 10 patients with osteosarcoma of the proximal humerus were treated by wide margin resection and reconstruction with pasteurized osteo-articular autologous bone graft. They were seven women and three men, between 9 and 30 years of age at the time of presentation (mean, 19 years) who were followed up for at least 3 years or until the patient's death (range, 6–84 months; mean, 48 months) (Table 1). No patients were lost to follow-up.

Chemotherapy was administered for all osteosarcomas. Neoadjuvant and adjuvant chemotherapy using methotrexate, ifosfamide, doxorubicin, and cisplatin were used for the treatment of osteosarcoma. Our concept of using preoperative radiotherapy is that, when a marginal or an inadequate wide margin can be predicted by preoperative imaging studies, preoperative radiotherapy is used for either low-grade or high-grade sarcoma. None of this series needed radiotherapy. The surgical stages of these patients according to the Musculoskeletal Tumor Society staging system [8] were stage IB (two patients) and stage IIB (eight patients) (Table 1). There were nine wide margins and one marginal margins. After resection, the surgical specimen was cleared of its soft tissue, the gross tumor, and intraosseous macroscopic portion of the tumor. The bone was kept in preoperatively heated saline at 60°C for 60 and then retrieved, kept in saline at room temperature for ~10 min, then put back at the original anatomic site and fixed with a plate, intramedullary nail, or screws with or without bone cement.

Autogenous graft was not used. All patients received intra-articular resection of the proximal humerus. For scintigraphic evaluation of the graft, the uptake is – if the uptake is less than the normal contralateral side or normal bone of the same limb, 0 if it is similar to the normal bone, and + if it is more than that of the normal bone as suggested by Ehara *et al.* [7,16].

The ethics committee of our institute approved the method of pasteurization of bone for skeletal reconstruction after resection of malignant bone tumors. Written consent was obtained from the patients included in this study. Results were calculated as percentage of the maximum score rating as described by Sabo *et al.* [27] for radiographic evaluation, and as described by Enneking *et al.* [9] for functional evaluation.

Results

Types of pasteurized bone grafts

Pasteurized permanent osteochondral graft was used in all patients, that was fixed with intra-medullary nail. Bone cement was used in two patients. The rotator cuff and the remnants of the capsule were repaired using nonabsorbable sutures. None of our patients required conversion surgery to a prosthetic replacement.

Radiographic study

Results according to the International Society of Limb Salvage graft evaluation score are shown in Table 2. Fusion scores were reduced markedly 1–3 months postoperatively. Callus formation was detected at 4–6 months (Fig. 1). The reduced scores were recovered by 4 months after surgery in most of the patients. Union occurred without additional treatment in nine patients (primary bone union). Conventional bone grafts were not needed. A 9-year-old girl was treated by

Table 1 Summary of the patients' data

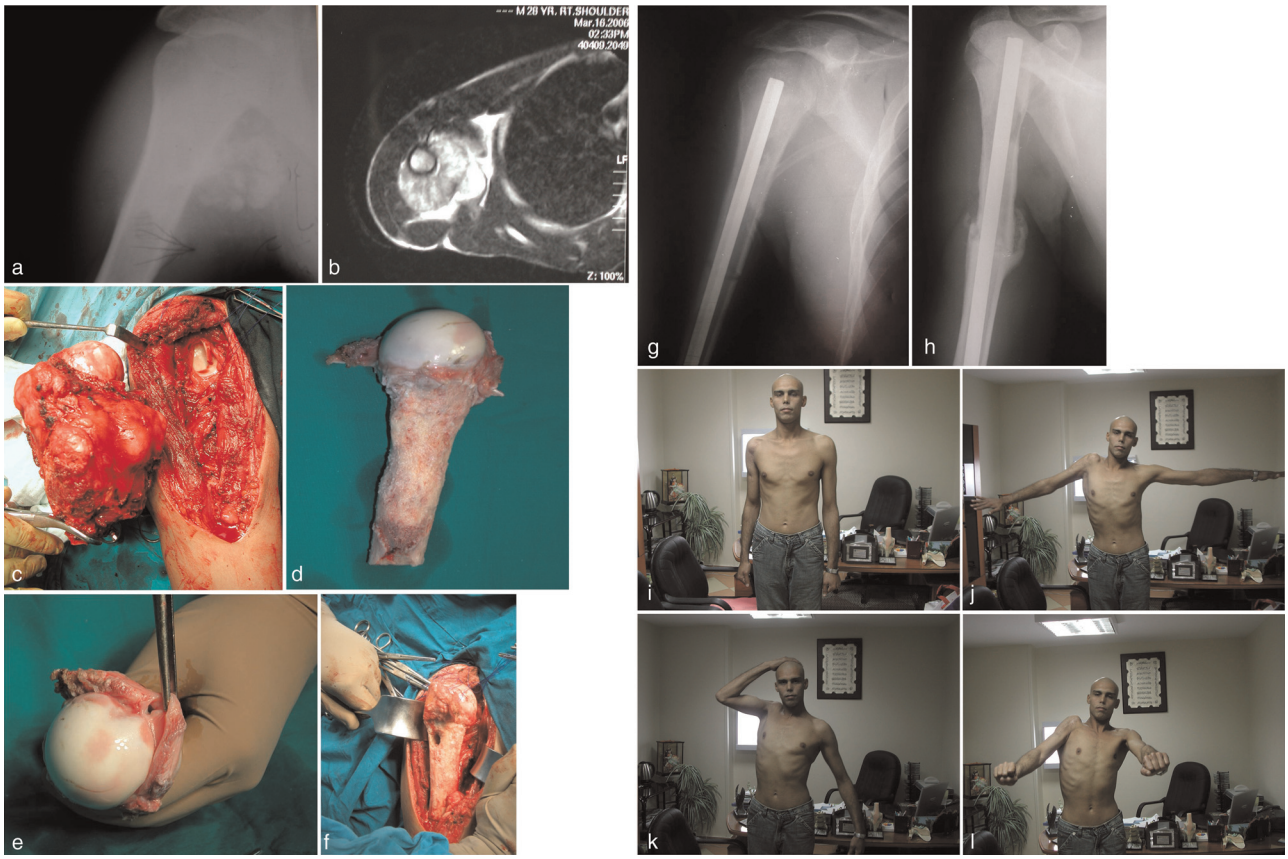
Patient number	Age (years)	Sex	Stage	Results	Follow-up (months)	Margin	Complications
1	30	M	IIB	CDF	84	Wide	None
2	20	F	IIB	DOD	36	Wide	None
3	23	F	IIB	CDF	70	Wide	None
4	16	F	IIB	NED	68	Wide	None
5	17	M	IIB	DOD	6	Wide	None
6	18	F	IIB	CDF	64	Wide	None
7	9	F	IB	CDF	60	Marginal	Massive resorption
8	18	F	IIB	DOD	20	Wide	None
9	17	F	IIB	NED	36	Wide	None
10	30	M	IB	CDF	36	Wide	Proximal protrusion of IMN

CDF, continous disaese free; DOD, death of disease; F, female; M, male; IMN, intra-medullary nail; NED, no evidence of disease.

Table 2 Type of grafts and scintigraphic, radiographic, and functional results

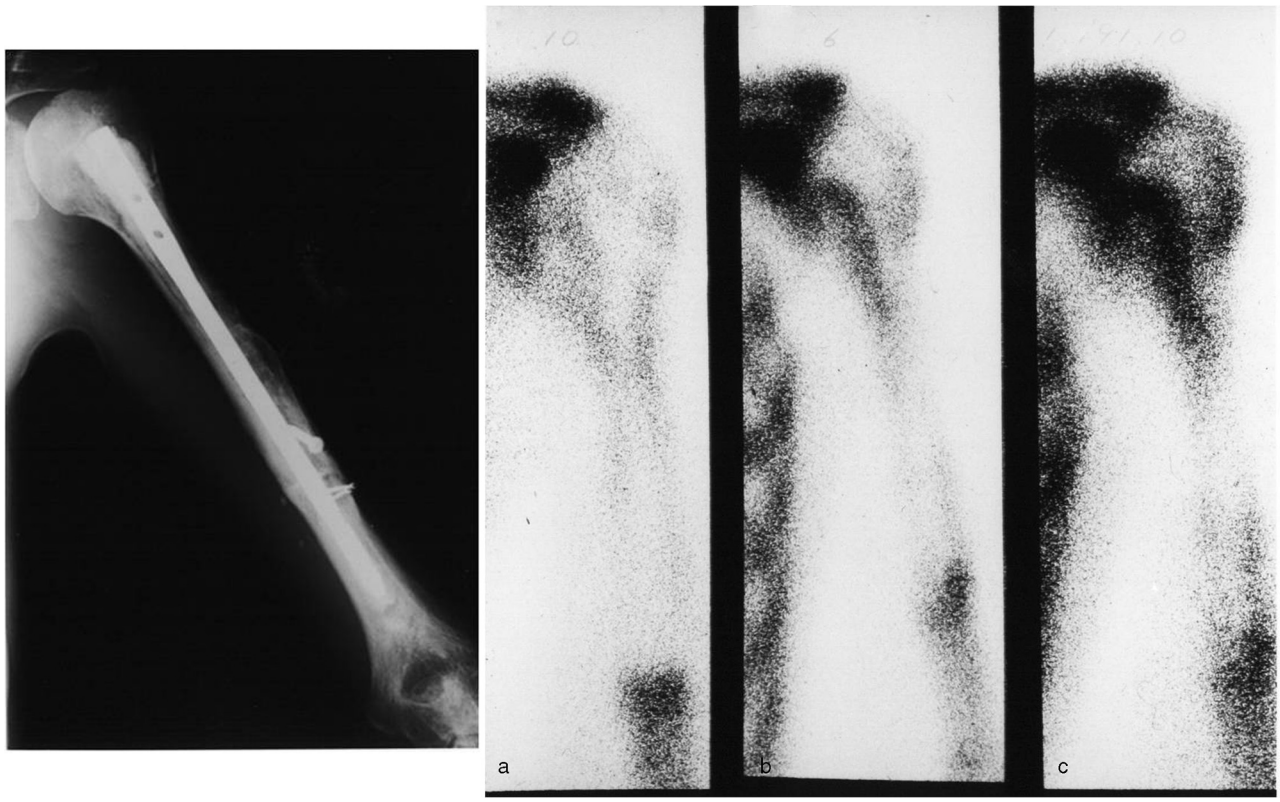
Patient number	Type of graft	Scintigraphic findings	Radiographic results score rating	Functional results score rating
1	Pasteurized osteochondral graft	+	30/32–94%	30/30 – 100%
2	Pasteurized osteochondral graft	0	28/32 – 88%	NA (DOD)
3	Pasteurized osteochondral graft	+	30/32 – 94%	30/30 – 100%
4	Pasteurized osteochondral graft	–	30/32 – 94%	30/30 – 100%
5	Pasteurized osteochondral graft	+	28/32 – 88%	NA (DOD)
6	Pasteurized osteochondral graft	0	28/32 – 88%	24/30 – 80%
7	Pasteurized osteochondral graft	NA	8/32 – 25%	23/30 – 76%
8	Pasteurized osteochondral graft	–	28/32 – 88%	NA (DOD)
9	Pasteurized osteochondral graft	0	28/32 – 88%	24/30 – 80%
10	Pasteurized osteochondral graft	+	30/32 – 94%	30/30 – 100%

Figure 1



Patient number 3: (a) plain radiography of his right proximal humerus and (b) axial T2 MRI showing the extent of the lesion.

Figure 2



Patient number 5: (a) his radiography 3 years after surgery showing solid union, good incorporation of the pasteurized osteo-articular graft.

osteochondral pasteurized bone graft developed gradual massive resorption of the pasteurized osteo-articular graft (patient 7) (Table 1). The overall union scoring rate was 70% as calculated from the maximum scoring rate. Infection was not encountered in this series of patients. Radiologically, six (60%) patients had complete incorporation of graft (>75% of The International Society of Limb Salvage score) and three (30%) patients had partial incorporation (<75% of The International Society of Limb Salvage score), while single patient had massive resorption of the graft (10%). The overall average radiographic evaluation rating was 84%. No local recurrence was detected.

Viability of the graft

According to the method of Ehara *et al.* [7,16] (Table 2), at the end of follow-up, nine of 10 patients had Tc scintigraphy and four had higher uptake than in normal bone; three had uptake similar to that in normal bone; and two had less uptake than in the normal bone (Fig. 2). The uptake progressed from the graft–host junction and margins of the cortical rims to the center of the grafted pasteurized bone.

Functional score

The overall mean of the Musculoskeletal Tumor Society [9] functional rating was 90% in the current patients (Table 2 and Fig. 1). In patients with humeral resection for which the deltoid muscle and rotator cuff were sacrificed for an adequate margin, a contracture developed in the shoulder; however, the patients had good stability and satisfactory ability to do activities of daily living because of elbow motion and intact hand function. When the deltoid muscle and rotators were preserved, the range of motion of the shoulder was more favorable.

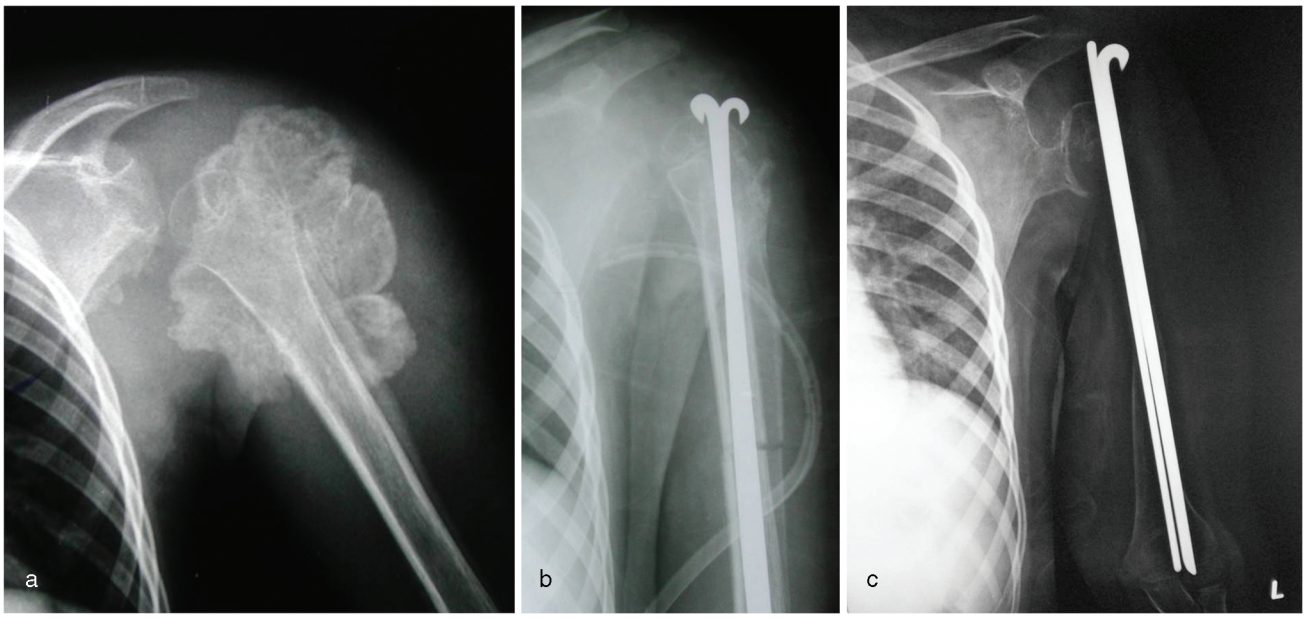
Oncologic results

There was no local recurrence in patients of this study including the single patient with marginal margins (Table 1). For out of the 10 patients with conventional osteosarcoma, five are continuously disease free, two have no evidence of disease, and three died of the disease. Three patients who died of the disease had stage IIB osteosarcoma.

Complications

Eight (80%) of the patients had no complications and achieved healing (Table 1). Fracture of the graft was not encountered in our patients. No patients needed autogenous iliac bone grafting for union to occur. Patient number 10 had revision of the internal fixation due to prominence of the intra-medullary nail at the shoulder 2 years after

Figure 3



Patient number 7: (a) her radiography at presentation.

reconstruction. This patient had excellent function at the end of follow-up. Infection was not encountered in this series of patients. No patients required conversion surgery to a prosthetic replacement. Although patient number 7 had 6 cm limb-length discrepancy, it was functionally insignificant. Massive resorption of the graft occurred in patient number 7 (Fig. 3); the age of the patient (9 years) may have contributed to this massive resorption. Fever or inflammatory findings suggesting immunologic reaction were not observed. No local recurrence was encountered in this series of patients.

Discussion

Several surgical procedures have been reported for the reconstruction of bony defects after tumor surgery. Prosthetic replacement is an option for limb salvage surgery, but loosening, breakage, and wear are encountered during long-term follow-up [4,13,28]. Allografts are also commonly used for reconstruction surgery but allografts require a large-scale bone bank system, and there are concerns of immunologic responses, transmission of diseases, religious and social circumstances, and high complication rate [2,12,13]. Therefore, an alternative is needed for the reconstruction surgery. Pasteurized autologous bone grafts are reasonable substitutes for allografts. Reintegration of the pasteurized bone graft used for reconstruction after resection of osteosarcoma in 10 patients is described. Inokuchi *et al.* [20] reported on three patients treated using pasteurized grafts for mandibular reconstruction after resection of malignant mandibular tumors. Two of their three patients had incorporation of the grafts [20]. The temperature used and duration of treatment in the current study were chosen because studies and clinical applications of the hyperthermia-treated bone proved the superiority of pasteurization of bone (heating at 60–65°C for 30 min) over other methods of heat treatment such as autoclaving or boiling [1,6,7,10,11,16,19–22,24,25]. Bone induction is produced by the response of the mesenchymal cells in the recipient bed to the BMP transferred from the bone implant; however in thermal exposures of 70°C and greater for 1 h, or irradiation sterilization, the biologic activity of the BMP is destroyed [23]. Pasteurization has a lethal effect on malignant cells while preserving sufficient biomechanical strength and bone-inducing property [1,6,7,10,11,16,19–22,24,25]. Moreover, Manabe [29] studied the intraosseous temperature curve changes in cadaveric human tibia and found that 30 min heating in a water bath at 60°C provides safe pasteurization. In the current study, the International Society of Limb Salvage system for evaluation of reintegration [15] showed that the scoring rate of the current study (84%) is better than that reported after the use of autoclaving [19] or extracorporeal irradiation (74%) [3], and excellent remodeling occurred after 6 months. This indicates that the bone-inducing property may not be disturbed by pasteurization, as suggested previously [1,6,7,10,11,16,19–22,24,25].

Graft resorption (a single patient) and proximal protrusion of the nail (a single patient) are seen in this series of patients. The age of the patient (9 years) may contribute to the resorption, while proximal migration of

the nail may be explained by some degree of graft collapse. The good functional results of this series may be explained by the non-weight-bearing nature of the proximal humerus, the rigid internal fixation, and coverage of the pasteurized bone graft with healthy vascular muscle tissue. Revision with a prosthesis was not done in any of our patients. The radiographic evaluation scores for nonunion, resorption, and consequent fracture seem comparable to those cited in other reports on allografts [2,12,13]. Although detailed prospective comparisons are needed, the advantages of a lack of immunologic responses to the pasteurized bone and the relative absence of deterioration caused by heating at a low temperature may have contributed to the satisfactory results in the rate of bone union and resorption. The Tc scan findings showed a remodeling process taking place in the transplanted grafts. Unfortunately, macroscopic and histologic examinations of the pasteurized bone were not available to document a remodeling process taking place in the transplanted grafts. However, change of the radiotracer uptake to a diffuse and homogeneous one is not a common feature for all the patients described here, and histologic studies that confirm that incorporation, perfusion, and replacement of the graft with the new bone were not available in this study. By 4–6 months in most of the patients callus formation was seen with partial cortication, and at 1 year complete incorporation of the grafts was seen in 60% of the patients and partial incorporation was present in 30% of the patients. The current findings are in line with those reported by Inokuchi *et al.* [20], Manabe *et al.* [22], and Ahmed [16]. In the current study, there were no local recurrences in patients with pasteurized bone grafts at a mean follow-up of 42 months, suggesting that pasteurization strongly proves that tumor sterilization was achieved. Rong *et al.* [24] reported this finding. Pasteurized bone graft is a new application for reconstruction in tumor surgery. The pasteurized bone is an avascular, nondenatured, best fitting, aseptic, and nonallergenic material. It may be best indicated when the structural integrity of the involved bone is still intact, however, it is contraindicated if there is significant loss of the structural integrity of the bone such as that caused by osteolytic tumors.As the number of patients and types of grafted bones in the current study are small and the complications must be reevaluated after a longer follow-up, and surgical indications must be defined for the use of pasteurized bone grafts in the treatment of musculoskeletal tumors. Heat treatment with pasteurization is a convenient method that showed satisfactory bone remodeling and union. The advantage of extracorporeal pasteurization may include avoidance of intraspecies infection such as HIV, viral hepatitis, allogenic reactions, and satisfactory bone remodeling. The current results suggest that pasteurization for proximal humeral reconstruction may be superior to other cell-lethal treatments for the autologous bone graft used for reconstruction surgery in patients with cancer.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interesting.

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