J Cancer Allied Spec 2024;10(1):3

Original Article

Complications and Outcome of Bone Sarcoma Patients with Limb Salvage using Liquid Nitrogen-treated Bone for Reconstruction

Muhammad Zoha Farooq*, Muhammad Bilal Shafiq, Sajid Ali, Ilyas Rafi

Department of Surgical Oncology, Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan

Received: 30 April 2023/Accepted: 08 September 2023

Correspondence:
Muhammad Zoha Farooq, Department of Surgical Oncology, Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan.
E-mail: zoha.farooq@gmail.com

Citation: Farooq MZ, Muhammad Bilal Shafiq, Sajid Ali, Ilyas Rafi. Complications and Outcome of Bone Sarcoma Patients with Limb Salvage using Liquid Nitrogen-treated Bone for Reconstruction. J Cancer Allied Spec [Internet]. 2024;10(1):1-8. https://doi.org/10.37029/jcas.v10i1.543

Funding: None.

Competing interest: The author(s) declare(s) that there is no conflict of interest.

Abstract

Introduction: The recommended treatment method for bone sarcoma is wide local excision and reconstruction to preserve limb function. Established methods of reconstruction are mega prosthesis or biological reconstruction. This study aimed to determine the complications and functional outcomes associated with limb salvage surgery using liquid nitrogen-treated bone. **Materials and Methods:** We retrospectively observed the short-term outcome of limb salvage surgeries where liquid nitrogen bone was used for reconstruction. A total of 15 patients underwent reconstruction with liquid nitrogen auto graft from January 2018 to December 2020. We used the free freezing method of liquid nitrogen treatment after wide local excision of sarcoma. We observed short-term outcomes after liquid nitrogen-treated bone reconstruction in limb salvage surgery. Survival of the auto grafts was recorded using the Kaplan–Meier method with a 95% confidence interval. **Results:** The mean follow-up was 19.83 ± 4.5 months. The mean musculoskeletal tumor society score was 62.4 ± 7.9%, while the average Toronto extremity score was 59.6 ± 5.7%. Three patients died during the study duration due to visceral metastasis. Skin necrosis and wound breakdown were major complications in 9 (60%) patients. Deep infection was observed in 4 (26.7%). Similarly, 4 (26.7%) patients had non-union at either the proximal or distal osteotomy site, while the average time of bone union in the rest of the patients was 6.3 ± 1.7 months. A total of 6 (40%) patients underwent reoperation after liquid nitrogen treatment, either due to infection or non-union at the osteotomy site. Recurrence was observed in 3 (20%) of patients. **Conclusion:** We observed a high complication rate with liquid nitrogen-treated autograft reconstruction. Vascularized fibula with liquid nitrogen-treated autograft or endoprosthesis should be encouraged.

Keywords: Bone sarcoma, cryotherapy, limb salvage, liquid nitrogen

Introduction

Primary bone sarcomas are uncommon, accounting for <0.2% of malignant neoplasms with distinct incidence patterns and clinical behavior.[1] Various age-specific histologic subtypes are reported with bimodal age distribution, with one peak seen in the adolescent and pediatric population while another occurs after 50 years of age.[2] Treating bone sarcoma in the pediatric and adolescent population

is challenging as the growing skeleton poses several changes, and they are more physically active than the adult population.[3]

In the past, radical treatment like amputation was preferred in adolescents. However, with the advancement of chemotherapy regimens, modern implant design, reconstructive options, and surgical techniques, limb salvage has become the standard for bone sarcoma.[4] However, multiple issues related to the adolescent and pediatric populations, such as mechanical failure, loosening, and durability, have also been reported.[5,6]

Several methods of biological reconstruction have been documented in literature irradiated, autoclaved, pasteurised, and frozen.[7-10] Extra-corporeal radiotherapy has the disadvantage of taking bone out of the theatre, while pasteurization results in the loss of both biological and mechanical properties of bone.[9,11] Liquid nitrogen-treated bone retains both osteoconductive and osteogenic properties.[12] Liquid nitrogen recycled bone is advantageous in selected reconstruction patients because it provides anatomically matched grafts and restores the bone length with sufficient strength.[10,13] Several studies documented better results with liquid nitrogen-treated bone reconstruction with the optimal bone union and functions, and it is the preferred reconstruction method in complicated cases.[14,15] However, complications and technical difficulties with using liquid nitrogen, such as handling, fractures, non-union, and wound-related problems, are well known.[16]

In developing countries like Pakistan, liquid nitrogen-treated bone for limb salvage is an economical option. However, late presentation with sizeable soft tissue components and long length of bone involvement constitutes a significant concern resulting in major resection of involved bone, over which the effect of liquid nitrogen has still not tested.[17] The objective of this study was to determine the complications and functional outcomes associated with limb salvage surgery using liquid nitrogen-treated bone.

Materials and Methods

This retrospective review was conducted in the Department of Surgical Oncology, Shaukat Khanum Memorial Cancer Hospital and Research Center, Lahore, Pakistan, after obtaining ethical approval from the institutional review board. Patients with primary malignant musculoskeletal tumors of the extremities who had undergone limb salvage surgery using a liquid nitrogen-treated bone graft from January 2018 to December 2020 with a minimum of 1-year follow-up were included in the study. Data were collected and entered on pre-designed forms.

All patients with limb sarcoma received neoadjuvant chemotherapy after initial presentation and workup. Patients with osteosarcoma underwent two cycles of Methotrexate, Doxorubicin, and Cisplatin (MAP) regimen neoadjuvant chemotherapy followed by surgery, then four cycles of adjuvant chemotherapy. Patients with Ewing sarcoma underwent six cycles of Vincristine, Doxorubicin, Cyclophosphamide alternating with Ifosfamide, Etoposide regimen neoadjuvant chemotherapy followed by surgery, then seven cycles of adjuvant chemotherapy.

We have used the free-frozen method of treating bone with liquid nitrogen as described by Tsuchiya *et al*.[10] Tumor-bearing bone was frozen in the liquid nitrogen for 20 min, then at room temperature, and distilled in water for 10 and 15 min. The frozen bone autograft was used for reconstruction. Bone graft or cement was used for mechanical support when necessary. The frozen bone was reconstructed using locking plates or intramedullary rods. We had performed intercalary resection in 14 patients, and one patient of distal femur underwent osteoarticular resection. To increase vascularity, free vascularized fibula graft was used in two patients of distal femur sarcoma along with liquid nitrogen-treated bone in two patients. Immediately after the surgery, patients were allowed range of movement exercise. Partial weight-bearing was allowed 2 months after surgery, and weight protection was continued until sufficient callus formation at the host graft junction occurred.

Patients were followed regularly for wound and functional score assessment with musculoskeletal tumor society (MSTS) score and Toronto extremity scoring system for operated limbs. In addition, patients underwent an X-ray chest for pulmonary metastasis and a computerized tomography (CT) scan of the involved limb for any recurrence and to assess bone union every 4 months. Based on the radiological findings of the CT scan limb, auto grafts that were conserved were deemed as "survived," and those that had been resorbed and were no longer functional were recorded as "died". Survival of autografts was recorded using the Kaplan–Meier method with a 95% confidence interval.

Statistical Package for the Social Sciences software (SPSS®) 23 was used for statistical analysis. Continuous variables as means ± Standard Deviation (SD) and analyzed using Student's t-test. Categorical data as numbers and percentages and analyzed using χ^2 or Fisher's exact test as appropriate in all the above statistical tools. $P \leq 0.05$ was considered to be statistically significant.

Results

Fifteen patients were reviewed; 11 (73%) were male and 4 (27%) were female. The mean age of patients was 13 years (range 7–22 years). The most common histopathology was osteosarcoma in 12 patients, while three had Ewing sarcoma. The distal femur was the common location for the oncological disease. It was seen in seven (46.7%) patients. Metastatic disease was not seen in any patients at the time of presentation.

Twelve (80%) patients were alive, while 3 (20%) died during the follow-up period. All of these patients had advanced disease with pulmonary metastasis. The mean follow-up of the remaining 12 patients was 19.83 ± 4.5 months.

Of the 12 surviving patients, in six patients, the liquid nitrogen grafts survived. Among those in whom the graft died, two patients developed a deep infection after surgery, one developed a recurrence, and three had a mechanical graft failure. Details are presented in Table 1. The mean survival of liquid nitrogen treated graft was 8.4 ± 2.09 (months) [Figure 1].

Eleven patients had a bone union (22 osteotomies), with the average union time being 6.9 ± 1.3 months. Four patients had non-union. Of these four (seven osteotomies), one patient underwent revised plating with bone graft to achieve union. Meanwhile, another patient presented with non-union at the distal femur proximal site and was replaced with endoprosthesis reconstruction. The remaining two patients ended up in amputation.

Post-operative histopathology of the sample showed closed margins (<5 mm) in four patients. However, none of these patients had recurrence during this study. Nonetheless, among the other patients, three developed recurrence, and the average time to recurrence after tumor resection was 6.7 ± 1.3 months. Two patients had recurrence after distal femur reconstruction with liquid nitrogen-treated bone, while one had recurrence at the proximal tibia.

Eleven (73%) patients developed complications after liquid nitrogen treatment, with superficial wound problems occurring in 8 (53.3%) patients, deep infection in 3 (20%) patients, and foot drop and wrist drop in one patient each.

The average MSTS score in liquid nitrogen-treated reconstruction was 62.4 ± 7.9%, while the average Toronto extremity score was 59.6 ± 5.7%. The functional score was lower in the proximal tibia, having a score of 51.7 ± 3.1, while the distal tibia had the highest functional score of 66.7%.

Discussion

The method of freezing bone with liquid nitrogen has been gaining popularity, especially in young individuals [Figure 2]. The outcome of liquid nitrogen-treated bone is better than allograft due to the lower rate of graft rejection.[18] This study assessed the complications and functional outcomes associated with limb salvage surgery using liquid nitrogen-treated bone.

Table 1: Details of patients treated with liquid nitrogen bone

Serial number	Age (years)	Cancer type	Tumor site	Follow-up (months)	Resection length (cm)	Wound complication	Recurrence	Bone union	Graft failure	Metastasis	Status
1	17	Osteosarcoma	Right proximal humerus	24	16	None	None	Not unite	Vascularized fibula	Lungs	-
2	14	Osteosarcoma	Left proximal tibia	20	28	Yes	Yes	Not unite	Amputation	-	-
3	22	Ewing	Left ulna diaphysis	14	23	None	None	Unite	-	-	-
4	7	Ewing	Right proximal tibia	26	20	Yes	None	Unite	Amputation	-	-
5	14	Osteosarcoma	Right distal femur	12	20	None	Yes	Not unite	-	Bone	Death
6	16	Osteosarcoma	Left distal tibia	29	20	None	None	Unite	-	-	-
7	11	Osteosarcoma	Left distal femur	03	19	None	None	Unite	-	Lung	Death
8	09	Ewing	Right proximal femur	22	20	None	None	Unite	-	Lung	Death
9	15	Osteosarcoma	Left proximal tibia	24	20	Yes	None	Unite	Amputation	Lung	Progression
10	13	Osteosarcoma	Right distal femur	14	25	Yes	No	Unite	Distal femur endoprosthesis	-	-
11	08	Osteosarcoma	Left distal femur	12	12	None	No	Unite	-	-	-
12	11	Osteosarcoma	Right proximal tibia	12	15	None	None	Unite	-	-	-
13	10	Osteosarcoma	Right distal femur	12	20	None	Yes	Unite	-	Lung and bone	Progression
14	11	Osteosarcoma	Left proximal femur	23	27	Yes	None	Unite	-	-	-
15	17	Osteosarcoma	Right distal femur	35	21	Yes	None	Not unite	Distal Femur Endoprosthesis	-	-

We used the free frozen method of liquid nitrogen treatment, which increases the osteotomy sites, and chances of complication. Shinozaki *et al*. described the pedicle freezing method of liquid nitrogen bone treatment, which decreased osteotomy site and had a better union rate as compared to free freezing.[19] The method of pedicle freezing is technically more difficult and data are limited regarding technical difficulties in using liquid nitrogen for pedicle freezing.[20]

Li *et al*., in their study, have shown a good functional outcome of liquid nitrogen-treated bone reconstruction with MSTS score of more than 80% and better outcomes in terms of gait in their patients.[21] On the contrary, Wisanuyotin *et al*. have documented low functional outcomes with an MSTS score of <70% with biological reconstructions.[22] The MSTS score of our patient was low (<60%). This may be due to delayed presentation and extensive resection of bone length in our population.

Comparable to our findings of an osteotomy union rate of 76% and a length of bone resection more than 209 ± 57.3 mm, the literature review found that the functional outcome had a bone union rate of more than 90% with a resected length of the bone 138.4 ± 60.39 mm. This suggests that there are more chances for non-union and complications with increased resection length.[15,23]

Therefore, to decrease the rate of non-union at the osteotomy site pedicle freezing method has been introduced which decreased the number of osteotomies and had better functional outcomes.[19] The use of vascularized fibula along with cryotherapy has shown better outcomes.[24] To increase the vascularity of bone, we have used vascularized fibula graft in two patients with Distal Femur Osteosarcoma numbered 11 and 12 in Table 1, which had shown early signs of union and decreased complications as compared to other patients.

Another method to increase bone union and decrease complications is to perform hemi cortical resection of diseased bone as described by Chen WM *et al*.[25,26] With this resection, one cortex remains in continuity, resulting in better structural support and more chances of liquid nitrogen-treated graft survival. However, this method is valid only for exophytic and small tumors. Furthermore, we did osteoarticular dissection in one patient with distal femur osteosarcoma and treated dissected bone and articular cartilage in liquid nitrogen. Higuchi T also has described knee preservation surgery using liquid nitrogen-treated bone with free freezing and pedicle method and documented the excellent functional outcome.[27] However, in our series, the developed dissociation of the epiphysis from the shaft at the level of physis results in impingement of the shaft with the patella and poor functional outcome.

Recurrence was seen in three patients, occurring from the soft-tissue component. The proximal tibia was the most common site of recurrence, probably due to the lack of soft tissue coverage around the proximal tibia and large soft-tissue component, making resection more difficult. The recurrence rate was slightly higher than described in the literature, which may be due to the extensive disease and large soft-tissue component in our patients.[13,21] Out of the 15 patients who underwent liquid nitrogen reconstruction in our series, 5 of them (33%) experienced distant metastasis. The lungs were the most commonly affected site. Similarly, Yoshihiro Arak's study also reported a distant metastasis rate of over 26% in patients who underwent liquid nitrogen treatment.[28] One of our patients had also developed metastasis in the contralateral tibia (solitary bone metastasis) along with pulmonary metastasis, which were rare findings not reported in the literature.

Skin sloughing and deep wound infection was the most common complication after liquid nitrogen reconstruction, which resulted in a delay in adjuvant chemotherapy, increased hospital stays, and poor outcomes. Skin sloughing was seen in more than 50% of our patients, which led to deep wound infection or required revision surgeries to cover the implant. Wound-related problems reported in

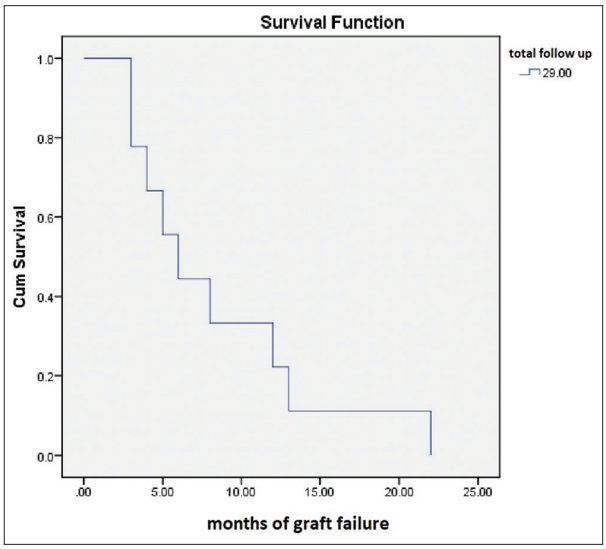

Figure 1: Survival curve for liquid nitrogen graft with mean survival of liquid nitrogen treated graft in our study is 8.4±2.09 (months)

Figure 2: Magnetic resonance imaging (MRI) axial (a) and coronal (b) views of 12-year-old male having osteosarcoma who underwent reconstruction with liquid nitrogen treated bone as shown in X-ray tibia anteroposterior (c) and lateral (d) views

the literature are comparable to our findings as one of the major complications after liquid nitrogen-treated bone reconstruction.[28,29] The absence of growth of immune cells and the hypothermic effect of liquid nitrogen on the surrounding structure may contribute to skin necrosis and infection. However, this effect of liquid nitrogen-treated sarcomas needs to be tested in future studies.[30]

We have observed the death of three patients, making the survival of bone sarcoma <80%, which is comparable to other studies, showing 3-year survival of 79%.[18] The weakest aspect of using liquid nitrogen bone grafts was that pathological examination was not performed, and the percentage of necrosis was not calculated. Because of this, the effectiveness of the pre-operative chemotherapy regimen cannot be determined, which resulted in the use of the same chemotherapy regimen in adjuvant cycles.[22] This may also result in an increase in recurrence and metastasis rate after liquid nitrogen reconstruction. This may also result in an increase in recurrence and metastasis rate after liquid nitrogen reconstruction. The limitation of this study was a small number of patients with a relatively shorter follow-up period. The small sample was due to the limited use of liquid nitrogen in only those patients in which adult-type mega prosthesis was not feasible. However, satisfactory reconstruction was achieved in all patients with minimal complications. The scope of our research encompasses a wide range of patients with varying medical conditions, including upper and lower limb injuries, as well as extra-articular and osteoarticular dissection. The distinct range of medical conditions may have an impact on the outcomes of our study.

Liquid nitrogen-treated autologous bone replantation is an alternate option for primary bone sarcomas in all age groups, especially in resource-limited environments, where allografts and end prostheses are difficult to obtain. However, it is a safe and reliable biological reconstruction method with a high complication rate, low healing end of osteotomy, and non-satisfactory postoperative function on short-term follow-up. Further, several aspects of this technique have yet to be thoroughly evaluated but may be helpful to consider for future studies to better treat this aggressive disease.

Acknowledgment

None.

References

1. Bone and Joint Cancer-Cancer Stat Facts. SEER; 2018. Available from: https://seer.cancer.gov/statfacts/html/bones.html [Last accessed on 2022 May 10].
2. Fletcher CD, Bridge JA, Hogendoorn PC, Mertens F. WHO Classification of Tumours. Soft Tissue and Bone Tumours. 4th ed. Lyon: IARC; 2013.
3. Closing the Gap: Research and Care Imperatives for Adolescents and Young Adults with Cancer. Report of the Adolescent and Young Adult Oncology Progress Review Group; 2006.
4. Han G, Bi WZ, Xu M, Jia JP, Wang Y. Amputation versus limb-salvage surgery in patients with osteosarcoma: A meta-analysis. World J Surg 2016;40:2016-27.
5. Smolle MA, Andreou D, Tunn PU, Leithner A. Advances in tumour endoprostheses: A systematic review. EFORT Open Rev 2019;4:445-59.
6. Grimer RJ, Aydin BK, Wafa H, Carter SR, Jeys L, Abudu A, et al. Very long-term outcomes after endoprosthetic replacement for malignant tumours of bone. Bone Joint J 2016;98-B:857-64.
7. Outani H, Takenaka S, Hamada K, Imura Y, Kakunaga S, Tamiya H, et al. A long-term follow-up study of extracorporeal irradiated autografts in limb salvage surgery for malignant bone and soft tissue tumors: A minimum follow-up of 10 years after surgery. J Surg Oncol 2020;121:1276-82.
8. Oike N, Kawashima H, Ogose A, Hatano H, Ariizumi T, Kaidu M, et al. Long-term outcomes of an extracorporeal irradiated autograft for limb salvage operations in musculoskeletal tumours: Over ten years' observation. Bone Joint J 2019;101-B:1151-9.
9. Lee SY, Jeon DG, Cho WH, Song WS, Kim BS. Are pasteurized autografts durable for reconstructions after bone tumor resections? Clin Orthop Relat Res 2018;476:1728-37.
10. Tsuchiya H, Wan SL, Sakayama K, Yamamoto N, Nishida H, Tomita K. Reconstruction using an autograft containing tumour treated by liquid nitrogen. J Bone Joint Surg Br 2005;87:218-25.
11. Puri A, Gulia A, Jambhekar N, Laskar S. The outcome of the treatment of diaphyseal primary bone sarcoma by resection, irradiation and re-implantation of the host bone: Extracorporeal irradiation as an option for reconstruction in diaphyseal bone sarcomas. J Bone Joint Surg Br 2012;94:982-8.
12. Takata M, Sugimoto N, Yamamoto N, Shirai T, Hayashi K, Nishida H, *et al*. Activity of bone morphogenetic protein-7 after treatment at various temperatures: Freezing vs. Pasteurization vs. Allograft. Cryobiology 2011;63:235-9.
13. Yamamoto N, Tsuchiya H, Tomita K. Effects of liquid nitrogen treatment on the proliferation of osteosarcoma and the biomechanical properties of normal bone. J Orthop Sci 2003;8:374-80.
14. Garg SK, Aggarwal P, Virk J, Punia RP, Dimri K, Jindal R Limb salvage using liquid nitrogen-treated tumour-bearing autograft: A single institutional experience of 10 patients. Indian J Orthop 2020;54:200-7.
15. Leung AS, Yeung MC, Yau RC, Ho KW, Shek TW, Lam AY. Case report on metastatic pelvic bone tumor treated with frozen autograft by liquid nitrogen. Int J Surg Case Rep 2021;82:105910.
16. Hindiskere S, Doddarangappa S, Chinder PS. What are the challenges and complications of sterilizing autografts with liquid nitrogen for malignant bone tumors? a preliminary report. Clin Orthop Relat Res 2020;478:2505-19.
17. Bhurgri Y, Bhurgri H, Pervez S, Kayani N, Usman A, Bashir I, et al. Epidemiology of soft tissue sarcomas in Karachi South, Pakistan (1995-7). Asian Pac J Cancer Prev 2008;9:709-14.
18. Smeland S, Bielack SS, Whelan J, Bernstein M, Hogendoorn P, Krailo MD, et al. Survival and prognosis with osteosarcoma: outcomes in more than 2000 patients in the EURAMOS-1 (European and American Osteosarcoma Study) cohort. Eur J Cancer 2019;109:36-50.
19. Shimozaki S, Yamamoto N, Shirai T, Nishida H, Hayashi K, Tanzawa Y, et al. Pedicle versus free frozen autograft for reconstruction in malignant bone and soft tissue tumors of the lower extremities. J Orthop Sci 2014;19:156-63.
20. Torigoe T, Tomita Y, Iwase Y, Aritomi K, Suehara Y, Oukubo T, et al. Pedicle freezing with liquid nitrogen for malignant bone tumour in the radius: A new technique of osteotomy of the ulna. J Orthop Surg (Hong Kong) 2012;20:98-102.
21. Li Y, Yang Y, Huang Z, Shan H, Xu H, Niu X. Bone defect reconstruction with autologous bone inactivated with liquid nitrogen after resection of primary limb malignant tumors: An observational study. Medicine (Baltimore) 2020;99:e20442.2.
22. Wisanuyotin T, Paholpak P, Sirichativapee W, Kosuwon W. Risk factors and outcomes for failure of biological reconstruction after resection of primary malignant bone tumors in the extremities. Sci Rep 2021;11:20444.
23. Zekry KM, Yamamoto N, Hayashi K, Takeuchi A, Higuchi T, Abe K, et al. Intercalary frozen autograft for reconstruction of malignant bone and soft tissue tumours. Int Orthop 2017;41:1481-7.
24. Erwin US, Cahyadi SD. Cryosurgery and vascularized fibular graft reconstruction in proximal tibia

osteosarcoma in young children: A case report. Int J Surg Case Rep 2021;89:106568.
25. Chen WM, Wu PK, Chen CF, Chung LH, Liu CL, Chen TH. High-grade osteosarcoma treated with hemicortical resection and biological reconstruction. J Surg Oncol 2012;105:825-9.
26. Shafiq MB. Limb salvage surgery in paediatric distal femur osteosarcoma with partial cortical resection and coraline silicon bone substitute-case report. J Pak Orthop Assoc 2021;33:41-5.
27. Higuchi T, Yamamoto N, Nishida H, Hayashi K, Takeuchi A, Kimura H, et al. Knee joint preservation surgery in osteosarcoma using tumour-bearing bone treated with liquid nitrogen. Int Orthop 2017;41:2189-97.
28. Araki Y, Yamamoto N, Hayashi K, Takeuchi A, Miwa S, Igarashi K, et al. Clinical outcomes of frozen autograft reconstruction for the treatment of primary bone sarcoma in adolescents and young adults. Sci Rep 2021;11:17291.
29. Wu PK, Chen CF, Chen CM, Cheng YC, Tsai SW, Chen TH, et al. Intraoperative extracorporeal irradiation and frozen treatment on tumor-bearing autografts show equivalent outcomes for biologic reconstruction. Clin Orthop Relat Res 2018;476:877-89.
30. Pritz T, Weinberger B, Grubeck-Loebenstein B. The aging bone marrow and its impact on immune responses in old age. Immunol Lett 2014;162:310-5.

Author Contributions

Conceived and designed the analysis: MBS, MZF, IR, and SA. Collected the data: MBS and SA. Contributed data or analysis tools: MBS, MZF, and IR. Performed the analysis: MZF and SA. Wrote the paper: MBS, MZF, IR, and SA.