Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

# Effect of donor age on release of bone morphogenetic protein in indigenously prepared demineralized freeze-dried bone allografts: An in vitro study

**Nasreen Ansari**
Maulana Azad Institute of Dental Sciences
**Arundeep Kaur Lamba**
Maulana Azad Institute of Dental Sciences
**Farrukh Faraz**
Maulana Azad Institute of Dental Sciences
**Shruti Tandon**
tandonshruti16@gmail.com
Maulana Azad Institute of Dental Sciences
**Zainab Kamal**
Maulana Azad Institute of Dental Sciences
**Zaid Kamal Madni**
**Archita Datta**
Maulana Azad Institute of Dental Sciences
**Anshika Maheshwari**
Maulana Azad Institute of Dental Sciences

**Research Article**

**Keywords:** Bone morphogenetic protein, donor age, demineralized freeze-dried bone allografts, osteoinductivity, tissue bank

**Posted Date:** June 29th, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-10047458/v1

**License:** This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

**Additional Declarations:** No competing interests reported.

**Version of Record:** A version of this preprint was published at Cell and Tissue Banking on November 15th, 2026. See the published version at https://doi.org/10.1007/s10561-026-10259-7.

# Abstract

**Introduction**: Demineralized freeze-dried bone allografts (DFDBA) have emerged as a promising graft material over the years, however, there is continued debate over the acceptable age range of donors from which the allograft is prepared and whether the effectiveness of this graft material is influenced by the donor age. Hence, this study was done to evaluate the effect of donor age on the release of bone morphogenetic protein-7 (BMP-7) within the indigenously prepared DFDBAs.

**Aim**: To evaluate the effect of donor age on the release of Bone Morphogenetic Protein-7 (BMP-7) in indigenously prepared Demineralized Freeze-Dried Bone Allografts (DFDBA).

Objectives:

• To quantify BMP-7 levels in DFDBA samples prepared from younger donors (aged 29–33 years) using ELISA.

• To quantify BMP-7 levels in DFDBA samples prepared from older donors (aged 55–60 years) using ELISA.

• To compare and statistically analyze BMP-7 concentrations between the two age groups.

**Method**: One hundred forty-four DFDBA samples were prepared using a standard protocol. Samples were divided into 2 groups containing 72 samples in each group. Group 1 samples were prepared from younger donors (age ranging from 29–33 years) and Group 2 samples were prepared from older donors (age ranging from 55–60 years). All samples were subjected to the BMP-7 extraction procedure and quantification was done using Enzyme-linked immunosorbent assay (ELISA).

**Results**: BMP-7 levels were found to be inversely related to donor age. Statistically significant higher values were obtained from samples belonging to Group 1 (29–33 years) as compared to Group 2 (55–60 years) with values of 201.705 ± 12.941 ng/g and 135.522 ± 10.974 ng/g respectively and p-value < 0.001.

**Conclusion**: The amount of BMP-7 was found to be lesser in grafts prepared from donors of the older age group, which may imply that donor age is a crucial factor to be considered in the preparation of bone allografts. This research contributes towards the preliminary phase for enhancing the quality of bone allografts. However, further in vivo studies with larger sample sizes can be done to form concrete evidence.


# Introduction

Demineralized freeze-dried bone allografts (DFDBAs) have emerged as promising graft materials owing to their osteoconductive and osteoinductive properties. DFDBA elicits its osteoinductive effect through the acid demineralization step during processing, which exposes growth factors such as bone morphogenetic proteins (BMPs) [1, 2]. However, contradictory evidence has been reported when DFDBA

is prepared from donors of different age groups, suggesting a possible decline in BMPs and growth factors during the graft processing stages. This wide variation in clinical outcomes following DFDBA application in various regenerative procedures has been attributed to several factors, including the presence of bone inductive proteins at subtherapeutic levels or in an inactive state. This variability in osteogenic response among commercially available DFDBAs may be influenced by multiple factors: donor age, particle size and shape, graft processing method, demineralization duration, and sterilization technique [3–5].

The ability of allografts to induce new bone formation can vary considerably between samples from the same tissue bank, depending on donor age. Numerous studies have reported differing clinical outcomes regarding new bone formation when using DFDBA samples procured from donors of different age groups [6, 7]. In light of these considerations, the present study was undertaken to produce safe and cost-effective allografts in accordance with internationally accepted standards, while also assessing the variability of BMP-7 levels among indigenously prepared graft samples based on donor age.

## Material and methods

The study included 144 DFDBA samples which were prepared using femur heads obtained from donors who underwent hip replacement surgery due to osteoarthritis or fractures, from the Department of Orthopaedics, Lok Nayak Hospital in New Delhi. Before the surgery, written informed consent was taken from all the donors and serological tests for HIV/AIDS, Hepatitis B, Hepatitis C, and Syphilis were performed. Patients over the age of 70, as well as those with a history of infection or prior irradiation at the donor site, connective tissue disorders such as rheumatoid arthritis, chronic use of steroids, tuberculosis, or malignancy, were excluded from the study **Fig. 1**. After obtaining the femur heads from the operation theatre, they were immediately transferred in sterile conditions to the Central Tissue Bank, Department of Periodontology, M.A.I.D.S. for storage at -80°C and further processing **Fig. 2**.

The femur heads were thawed at room temperature and were cut into small pieces followed by conversion into fine bone particles using a bone blender. One hundred forty-four DFDBA samples were prepared (multiple graft samples from each donor were prepared) and processed using a standard protocol that involved cleaning, defatting, demineralization, and lyophilization procedures [8]. Samples were divided into 2 groups containing 72 samples in each group. Group 1 samples included those which were prepared from donor age ranging from 29–33 years and Group 2 samples were prepared from donor age ranging from 55–60 years. All samples were sealed and packed under sterile conditions **Fig. 3.**

BMP-7 was extracted according to a standard protein extraction technique advocated by Sampath TK et al. 1984 and Pietrzak et al. 2006 [9].

From each sample, 250 mg of bone allograft was measured using Electronic Balance machine (Mettler Toledo, Columbus, United States) and was stirred with 5 mL of 4 M guanidine hydrochloride (GuHCl)/0.05 M Tris-HCl for 24 hours using a Magnetic stirrer (Thermo Scientific Cimarec, Germany) at

4°C. All samples were centrifuged at 4°C for 15 minutes at 2,800 RPM in temperature-controlled centrifugation machine (Eppendorf SE, Germany 5920 R), followed by collection of supernatants, which was later dialyzed using a 12 KDa molecular weight cutoff membrane, for 15 hours at 4°C. The extracted BMP-7 was quantified using ELISA (Human BMP-7 ELISA kit, G- Biosciences, USA) **Fig. 4**.

# Statistical Analysis

The Shapiro-Wilk test was performed to assess the normality of data distribution. Data from both groups were found to be normally distributed. Levene's test for equality of variances was conducted to assess homogeneity of variance prior to inferential testing. An independent samples t-test (unpaired t-test) was used to compare BMP-7 concentrations between the two groups. The level of statistical significance was set at p-value less than 0.05.

## Results and Observation

The mean BMP-7 concentration in Group 1 (29–33 years) was 201.705 ± 12.941 ng/g, which was significantly higher than Group 2 (55–60 years) at 135.522 ± 10.974 ng/g. The difference between the two groups was statistically significant [$t(142) = 33.10$, $p < 0.001$], as shown in **Table 1 and Fig. 5.**

## Discussion

The use of bone grafts and substitutes in periodontics for various regenerative procedures has significantly expanded in recent years. Through its osteoconductive and osteoinductive properties, bone allografts can successfully be employed in periodontal regeneration depending on their type and the processing techniques used to prepare them.

Donor age has been shown to influence the composition and biomechanical properties of bone tissue, which, in turn, could affect the binding, release, and bioactivity of growth factors such as BMPs [10]. Previous research has shown that BMP levels vary depending on donor age, gender, and processing procedures. [11]

In the present study, to determine whether donor age and the amount of extractable growth factors are related or not, DFDBA samples were prepared from donors with age ranging from 29 to 60 years and BMP-7 was extracted using guanidine HCl which has been proven to be the most efficient among other methods of protein extraction [12]. The extracted BMP-7 was quantified using ELISA and it was found to be inversely related with donor age with statistically significant higher values seen in allograft samples obtained from young donors (29–33 years) as compared to donors of older age group (55–60 years). These results were consistent with study which correlated the levels of extractable BMPs from allograft and the donor age [13].

Experimental studies in animal models reported an inverse correlation with the ability of DFDBA to induce new bone formation and the donor age [14–15]. The possible reason for donor age related

decrease in osteoinductivity of allografts may be attributed to the gradual decline in matrix morphogenetic activity of bone and reduced bone mass associated with old age [16]. However, contrasting results were reported by Jergesen et al., who demonstrated higher osteoinductive potential of demineralized bone powder prepared from middle-aged adult rats compared with that prepared from either prepubertal or young postpubertal animals [17]. Nyssen-Behets et al. demonstrated, using a human demineralized bone matrix (DBM) athymic mouse assay, that there was no difference in the amount of new bone formed from DBM prepared from donors aged 18–46 years compared with those aged 62–90 years [18].

Panahipour et al. (2021) demonstrated that standard allograft processing steps, including defatting, demineralization, virus inactivation, and sterilization, preserve biologically active growth factors within DBM. The study confirmed that TGFβ1, a member of the same superfamily as BMP-7, remains detectable and functionally active in processed DBM preparations via ELISA-based quantification. These findings validate the use of ELISA for growth factor quantification from bone allograft extracts, as employed in the present study. Furthermore, this supports the premise that donor-related biological variables, such as age, may significantly influence the growth factor content retained within processed bone allografts [19].

Alaribe et al. (2016) evaluated the osteoinductive activity of DBM from donors of varying ages and reported variable osteoinductive capacity across age groups, with DBM from donors aged 45–55 years yielding the most favorable outcomes. BMP-2 was found to stimulate alkaline phosphatase activity and osteoblastic differentiation in C2C12 cells, confirming the age-dependent variation in osteoinductive potential of DBM. These findings are in concordance with the present study, where significantly higher BMP-7 levels were observed in younger donors (29–33 years) compared to older donors (55–60 years) [20].

Ansari et al. (2025) investigated the effect of demineralization time on BMP-7 levels in indigenously prepared DFDBA from the Central Tissue Bank, MAIDS, New Delhi, using the same ELISA-based quantification methodology. The study reported significantly higher BMP-7 concentrations in grafts demineralized for 18 hours (135.609 ± 11.943 ng/g) compared to those demineralized for 90 minutes (95.442 ± 9.226 ng/g), confirming that demineralization time critically influences BMP bioavailability. These findings provide methodological validation for the present study, which utilized the same tissue bank protocol and ELISA kit (G-Biosciences, USA) to quantify BMP-7 from DFDBA samples [21].

# Conclusion

The amount of bone morphogenetic proteins decreases with an increase in donor age, rendering bone allografts to be less osteoinductive as indicated by significantly reduced concentration of BMP-7 in DFDBA prepared from older donors. Natural variability of human donors related to age and gender is reflected in the bone induction ability of grafts and few DFDBA batches may yield better results than others even when identical procedures have been used to prepare them.

Further studies with greater sample sizes are required to formulate concrete evidence. Furthermore, future research endeavours could include donors of various ages and genders to provide substantial verification of DFDBA's osteoinductive effects. In general, tissue banks should prefer donors belonging to younger age groups for the preparation of allografts. Also, ELISA testing for BMPs can be used as a measure to characterize the osteoinductive ability of bone allografts and tissue banks may consider it as a routine quality-control test.

# Declarations

### Compliance with ethical standards

### Conflict of interest

The authors state that they do not have any conflicts of interest.

### Ethical approval

This article contains no animal studies conducted by any of the authors. All procedures involving human participants were carried out in accordance with the institutional research committee's ethical standards, as well as the 1964 Helsinki Declaration and its subsequent amendments.

Ethical approval was obtained from the Institutional Ethics Committee, Maulana Azad Institute of Dental Sciences, New Delhi (IEC Ref No. ECR/1767/inst / DEL/2020.)

### Funding

This study did not receive any funding from public, commercial, or non-profit organizations.

### Informed consent

All human tissue donors provided informed consent

# References

1. Urist MR. Bone induction by autoinduction. Science 1965; 150: 893–899
2. Huang YH, Polimeni G, Qahash M, Wikesjo ME. Bone morphogenetic proteins and osseointegration: Current knowledge-future possibilities. Periodontology 2000 2008; 47:206–23
3. Fucini SE, Quintero G, Gher ME, Black BS, Richardson AC. Small versus large particles of demineralized freeze-dried bone allografts in human intrabony periodontal defects. J Periodontol. 1993;64(9):844-7. doi: 10.1902/jop.1993.64.9.844. PMID: 8229619.
4. Song KX, Ji SL, Zhao YJ, et al. Effects of demineralization mode and particle size of allogeneic bone powder on its physical and chemical properties. Cell Tissue Bank. 2022;10.1007/s10561-022-10025-5.

5. Stevenson S. The effect of processing and low dose irradiation on cortical bone grafts. Clin Ortho Relat Res 2000;375: 275–285
6. Lohmann CH, Andreacchio D, Köster G, Carnes DL Jr, Cochran DL, Dean DD, Boyan BD, Schwartz Z. Tissue response and osteoinduction of human bone grafts in vivo. Arch Orthop Trauma Surg. 2001;121(10):583 – 90. doi: 10.1007/s004020100291. PMID: 11768641.
7. Schwartz Z, Mellonig JT, Carnes DL Jr, de la Fontaine J, Cochran DL, Dean DD, Boyan BD. Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation. J Periodontol. 1996;67(9):918 – 26. doi: 10.1902/jop.1996.67.9.918. PMID: 8884650.
8. Kamal Z, Lamba AK, Faraz F, Tandon S, Datta A, Ansari N, Madni ZK, Pandey J. Effect of gamma and Ultraviolet-C sterilization on BMP-7 level of indigenously prepared demineralized freeze-dried bone allograft. Cell Tissue Bank. 2024;25(2):475–484. doi: 10.1007/s10561-023-10103-2. Epub 2023 Aug 14. PMID: 37578672.
9. Sampath TK, Reddi AH. Distribution of bone inductive proteins in mineralized and demineralized extracellular matrix. *Biochem Biophys Res Commun*. 1984;119(3):949–954. doi:10.1016/0006-291x(84)90865-9
10. Khan SN, Cammisa FP Jr, Sandhu HS, Diwan AD, Girardi FP, Lane JM. The biology of bone grafting. J Am Acad Orthop Surg. 2005 Jan-Feb;13(1):77–86. PMID: 15712985.
11. Traianedes K, Russell JL, Edwards JT, Stubbs HA, Shanahan IR, Knaack D. Donor age and gender effects on osteoinductivity of demineralized bone matrix. J Biomed Mater Res B Appl Biomater. 2004;70(1):21 – 9. doi: 10.1002/jbm.b.30015. PMID: 15199579.
12. Wildemann B, Kadow-Romacker A, Pruss A, Haas NP, Schmidmaier G. Quantification of growth factors in allogenic bone grafts extracted with three different methods. Cell Tissue Bank. 2007;8(2):107–114. doi:10.1007/s10561-006-9021-0
13. Schwartz Z, Somers A, Mellonig JT, Carnes DL Jr, Dean DD, Cochran DL, Boyan BD. Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation is dependent on donor age but not gender. J Periodontol. 1998;69(4):470-8. doi: 10.1902/jop.1998.69.4.470. PMID: 9609378.
14. Piattelli A, Scarano A, Corigliano M, Piattelli M. Comparison of bone regeneration with the use of mineralized and demineralized freeze-dried bone allografts: a histological and histochemical study in man. Biomaterials. 1996;17(11):1127–1131.
15. Pietrzak WS, Woodell-May J, McDonald N. Assay of bone morphogenetic protein-2, -4, and – 7 in human demineralized bone matrix. J Craniofac Surg. 2006;17(1):84–90. doi: 10.1097/01.scs.0000179745.91165.73. PMID: 16432413
16. Honsawek S, Powers RM, Wolfinbarger L. Extractable bone morphogenetic protein and correlation with induced new bone formation in an in vivo assay in the athymic mouse model. Cell Tissue Bank. 2005;6(1):13–23. doi: 10.1007/s10561-005-1445-4. PMID: 15735897.
17. Jergesen HE, Chua J, Kao RT, Kaban LB. Age effects on bone induction by demineralized bone powder. Clin Orthop Relat Res. 1991;(268):253–9. PMID: 2060217.

18. Nyssen-Behets C, Delaere O, Duchesne PY, Dhem A. Aging effect on inductive capacity of human demineralized bone matrix. Arch Orthop Trauma Surg. 1996;115(6):303–6. doi: 10.1007/BF00420320.
19. Panahipour, L., Omerbasic, A., Nasirzade, J., & Gruber, R. (2021). TGF-β Activity of a Demineralized Bone Matrix. *International Journal of Molecular Sciences*, *22*(2), 664.
20. Alaribe FN, Razwinani M, Maepa MJ, Bierman F, Motaung SC. Osteoinductive Activity of Selected Demineralized Bone Matrix Products from Donors of Different Ages. Iran Biomed J. 2016 Jul 25:Pii-IBJ-A-10-1994-1. Epub ahead of print. PMID: 27452604.
21. Ansari N, Faraz F, Lamba AK, Tandon S, Kamal Z, Madni ZK. Effect of Demineralization Time on the Release of Bone Morphogenetic Protein in Indigenously Prepared Demineralized Freeze-Dried Bone Allografts: A Comparative In Vitro Study. Cureus. 2025;17(5):e84037. doi: 10.7759/cureus.84037. PMID: 40519500; PMCID: PMC12162367.

## Table 1

Table 1 is available in the Supplementary Files section.

## Figures

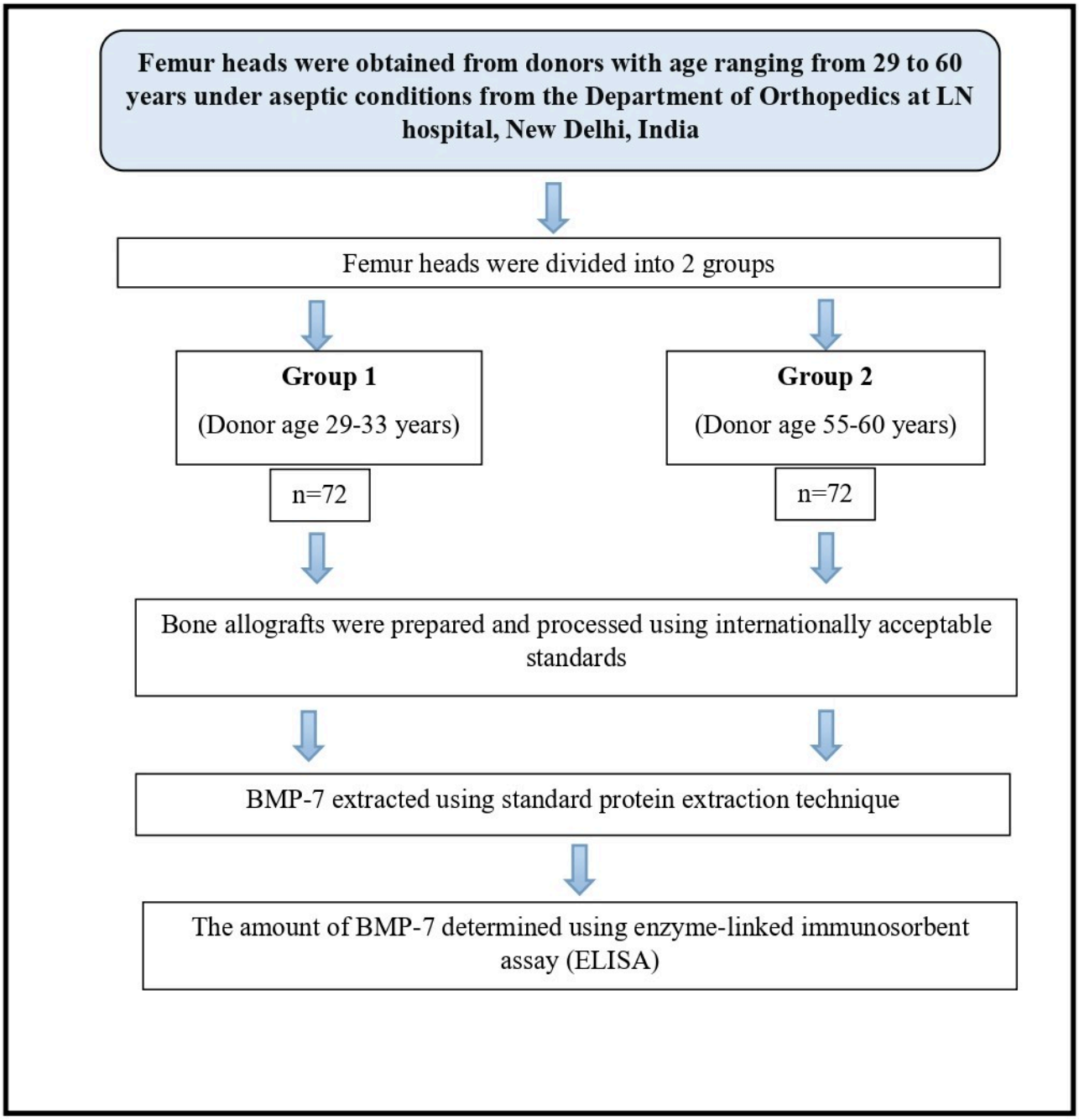



**Fig. 1. Study design**

## Figure 1

See image above for figure legend

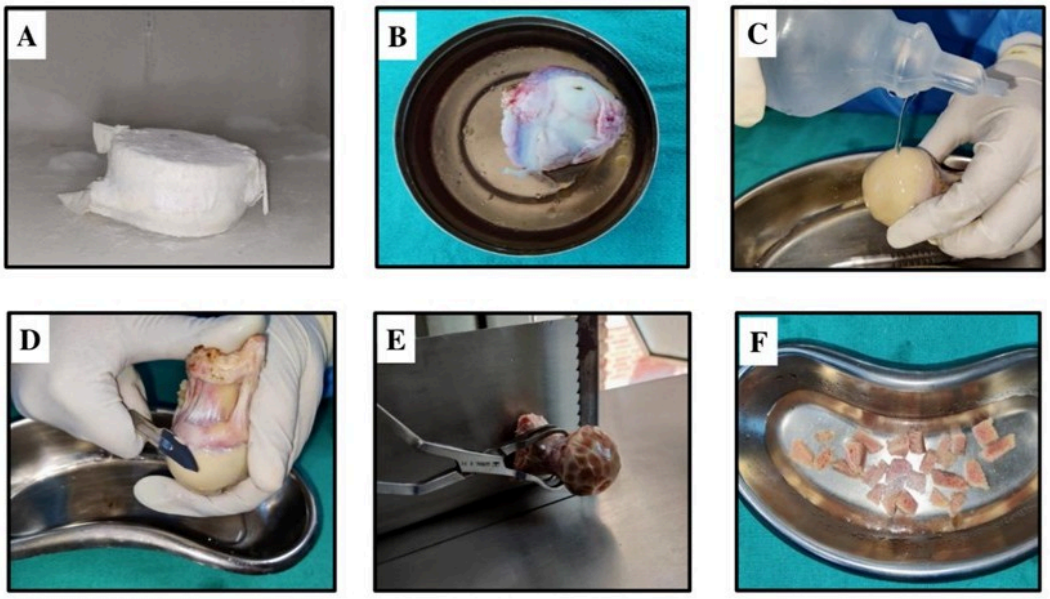



**Fig. 2. Processing of Demineralized freeze-dried bone allograft**:

A. Femur head in deep freezer under sterile condition. B. Thawed Femur head. C. Washing step with saline. D. Removal of soft tissue and cartilage. E-F. Cutting of femur head with bone cutter into small blocks.

## Figure 2

See image above for figure legend



**Fig.3.** Prepared DFDBAs sealed and packed under sterile conditions

## Figure 3

See image above for figure legend




**Fig. 4 BMP- 7 Extraction Procedure:** (a) Weighing of graft particles, (b) Stirring of grafts with extraction buffer using a Magnetic stirrer, (c) Centrifugation (All samples were at 4°C for 15 minutes at 2,800 RPM in temperature-controlled centrifugation machine (Eppendorf SE, Germany 5920 R)

(d) collection of supernatants

(e) Dialysis of supernatant for 15 hours at 4°C.

(f)The extracted BMP-7 was quantified using ELISA (Human BMP-7 ELISA kit, G- Biosciences, USA)

## Figure 4

See image above for figure legend

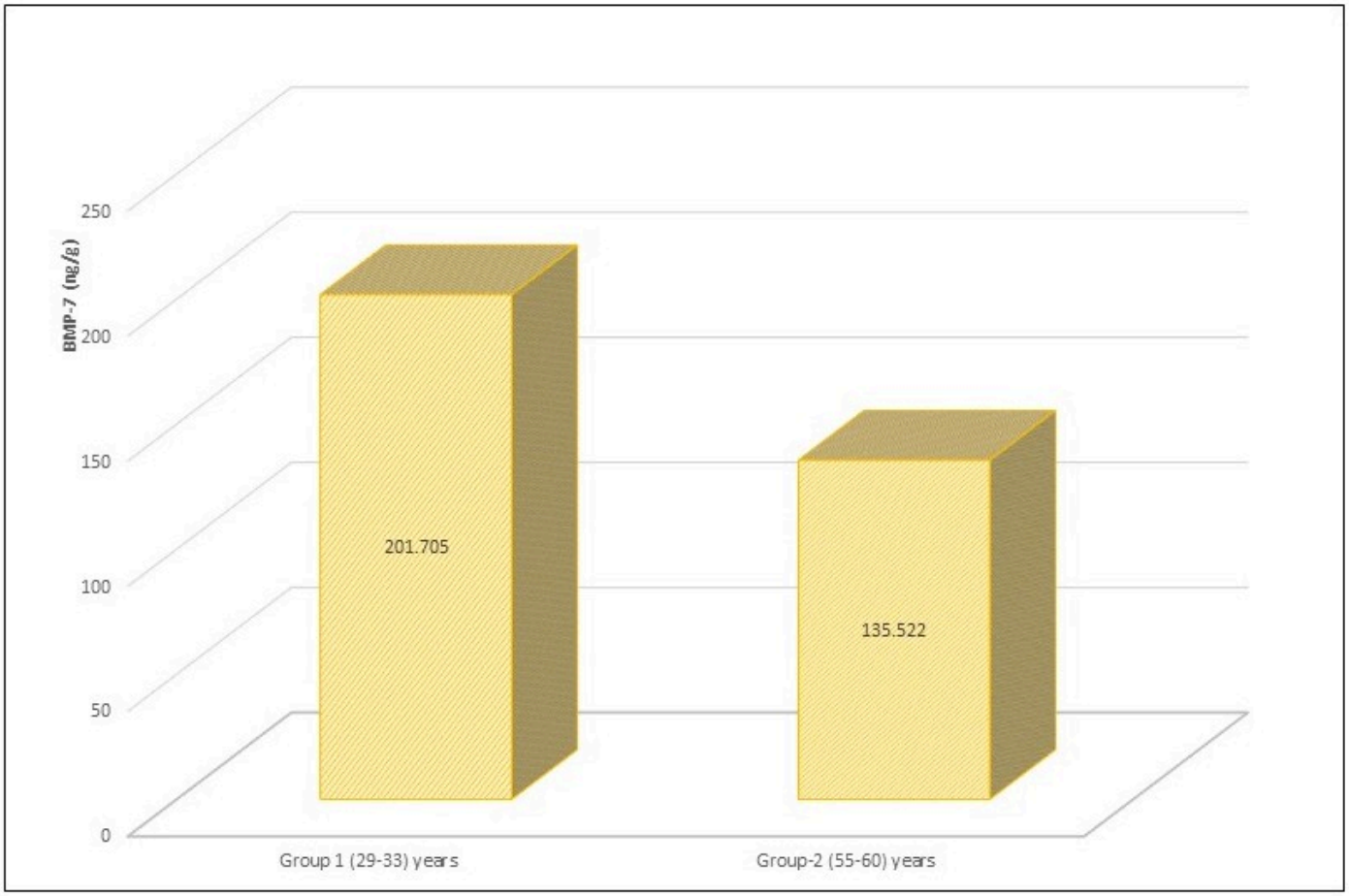



**Fig. 5** Comparison of mean BMP-7 concentration of different groups

**Figure 5**

See image above for figure legend

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- table1.docx