

Research Article

Evaluation of Mandibular Bone Healing in Wistar Rat: Relevance of Serum Biochemical Markers and Surface Bone Mineral Density

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Abstract

Introduction-Mandibular bone healing is a complex and crucial process in maxillofacial surgery. This study aims to explore the relevance of serum biochemical markers in monitoring mandibular bone healing in Wistar rats. Materials and Methods-An experimental study was carried out in 24 Wistar rats for three months, from February to April 2024, to demonstrate the relevance of serum biochemical markers (alkaline phosphatase, calcium, phosphorus) in monitoring mandibular bone healing. Biochemical assays were performed weekly and bone mineral density (BMD) measurements were taken every two weeks. Data were analyzed using GraphPad Prism 8.0.1 software, and correlation was assessed using Pearson's coefficient (r). Results-In female rats with dental extraction, alkaline phosphatase showed a strong positive correlation with dental BMD ($r=1.0$ at week 4), while phosphorus exhibited a perfect negative correlation ($r=-1.0$) at the same timepoint. In males, alkaline phosphatase and calcium demonstrated strong positive correlations with dental BMD ($r=0.8$ and $r=0.9$ respectively during week 4). In rats with symphyseal drill holes, both genders showed moderate to strong positive correlations between alkaline phosphatase/calcium and symphyseal BMD ($r=0.6-0.8$), with phosphorus showing variable correlations across weeks. Conclusion-Biochemical markers such as alkaline phosphatase and calcium, together with bone mineral density measurements, offer effective monitoring of bone healing and could optimize clinical protocols for patients with bone problems.

Keywords

Mandibular Bone Healing, Serum Biochemical Markers, Surface Bone Mineral Density, Wistar Rat, Evaluation

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1. Introduction

Bone is a dynamic living tissue that continuously adapts to mechanical stresses and responds to various lesions. Bone healing represents a complex biological process that involves the coordination of multiple cells, tissues, and biochemical factors [1, 2]. The mandible is particularly prone to fractures and bone diseases, making it essential to understand the mechanisms of bone healing in this region [3].

Biochemical markers play a crucial role in initiating and regulating the remodeling phase after bone injury. These markers can be categorized into those derived from bone formation (alkaline bone phosphatase, osteocalcin, C-terminal propeptide of type I procollagen, N-terminal propeptide of type I procollagen) and those from bone resorption (tartrate-resistant acid phosphatase, hydroxyproline, hydroxylysine glycosides, and pyridinolines) [4].

For this study, we selected alkaline phosphatase, calcium, and phosphorus as they represent key indicators of bone metabolism that are readily measurable and clinically relevant. Alkaline phosphatase is particularly important as it is directly involved in osteoblast activity and matrix mineralization, while calcium and phosphorus are the primary mineral components of bone tissue.

Bone Mineral Density (BMD) serves as a valuable indicator in the diagnosis of osteoporosis, a condition characterized by decreased bone density and increased fracture risk [5]. However, BMD is not merely a marker of long-term bone health; it can also reflect dynamic changes during bone remodeling processes related to injuries or surgical interventions [6, 7]. Recent studies have highlighted the importance of monitoring BMD changes during the healing process, as these can provide critical information about the rate and quality of bone regeneration [7-9].

Despite the importance of these parameters, few studies have evaluated mandibular bone healing using both serum biochemical markers and BMD measurements. Recent research by Zheng et al. (2021) and Kim et al. (2020) has begun to explore these relationships, but comprehensive analyses of marker-BMD correlations in mandibular healing models remain limited [9, 10]. Therefore, this study aims to determine, through an experimental approach, the activity of bone remodeling markers and BMD during healing in Wistar rats subjected to dental extractions and symphyseal drilling lesions.

2. Materials and Methods

2.1. Type and Location of Study

This was a prospective experimental study with a descriptive aim conducted over a period of 3 months.

2.2. Study Duration/Period

The study was carried out over a period of three months, from February to April 2024, with a follow-up time of 45 days.

2.3. Population Studied

The study population consisted of 24 Wistar rats that met the inclusion criteria (age of 8 weeks with a minimum weight of 150g) and exclusion criteria (death or weight loss exceeding 10% of initial weight). The rats were randomly divided into three groups of 8 rats each (4 females and 4 males), according to the absence of lesions or the type of lesions to be induced:

- 1) First group (G1): Control group without any intervention
- 2) Second group (G2): Rats undergoing extraction of the lower incisor
- 3) Third group (G3): Rats receiving a 1.6mm bone drill hole at the mandibular symphysis level

2.4. Data Collection

2.4.1. Biochemical Analysis

Blood samples were collected from all 24 rats in the morning on an empty stomach by inserting a glass micro-hematocrit capillary tube into the retro-orbital area. The blood was collected in dry tubes, and analyses were performed on the same day.

The following biochemical parameters were measured:

- 1) Calcium and inorganic phosphorus using the colorimetric method with Arsenazo III reagent for calcium and ammonium molybdate for phosphorus
- 2) Alkaline phosphatase (ALP) using a kinetic technique with BIOLABO commercial kit, which measures enzymatic activity based on the hydrolysis of p-nitrophenylphosphate

These measurements were taken for all three groups before the start of the experiment (W0) and then weekly until week 6 (W6).

2.4.2. Radiological Assessment

Rats were scanned using a 16-bar HITACHI SUPRIA scanner every two weeks after the interventions. Prior to scanning, each anesthetized rat was attached to a perforated Plexiglas plate and secured with string to ensure proper positioning and reduce movement artifacts.

The scanner measured the linear attenuation coefficient of the X-ray beam for each volume element (voxel) and calculated the degree of mineralization from this attenuation coefficient. Two-dimensional sections were obtained using a

virtual 1.25 mm section plane through the mandibular symphysis.

The following parameters were measured:

- 1) Three-dimensional geometry
- 2) Bone mineral density in regions of interest (alveolar-dental region of the lower central incisor and mandibular symphysis)
- 3) Size of bone defects on axial and sagittal sections

2.5. Study Variables

The variables of interest in this study included:

- 1) Animal weight
- 2) Activity of alkaline phosphatase, calcium, and inorganic phosphorus
- 3) Surface bone mineral density at the alveodental level
- 4) Surface bone mineral density at the mandibular symphysis level

2.6. Statistical Analysis

Data analysis was performed using GraphPad Prism version 8.0.1 software. Results for quantitative variables were expressed as mean \pm standard error of the mean. The correlation between variables of interest was evaluated using Pearson's coefficient (r), with the following interpretation scale:

- 1) $|r| = 0$: No correlation

2) $0 < |r| < 0.3$: Weak correlation

3) $0.3 \leq |r| < 0.5$: Moderate correlation

4) $0.5 \leq |r| < 0.7$: Strong correlation

5) $0.7 \leq |r| < 0.9$: Very strong correlation

6) $|r| \geq 0.9$: Near perfect correlation

7) $|r| = 1$: Perfect correlation

2.7. Ethical Considerations

This study received ethical clearance (N:854 CI-ER/UY1/FMSB/VDRC/DAASR/CSD). The research adhered to the Marshall Hall Principles, the Principles of Laboratory Animal Care, and the 3R Rule (Replacement, Reduction, Refinement).

3. Results

3.1. Biochemical Data

Notable trends include a progressive increase in alkaline phosphatase levels in both experimental groups (GII and GIII) compared to the control group, with particularly marked elevations after week 4. Calcium levels also showed consistent increases across all groups, while phosphorus demonstrated more variable patterns between groups and sexes.

Table 1. Summary of mean values for biochemical parameters obtained in our experimental groups.

		Time (in weeks)											
Biochemical parameters	Experi- mental Groups	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6	
		Females	Males	Females	Males	Females	Males	Females	Males	Fe- males	Males	Females	Males
Alkaline phosphatase (UI/L)	G I (CON)	80.25	88.75	89.75	147.75	101.5	144.75	120.25	140.75	89	169.33	200.33	337
	G II (DE)	82.5	56.75	83.75	84	134.67	135.5	289.33	155.25	387	177.75	437	336.25
	G III (DH)	64	135.67	116.33	177	154.75	211.25	183.67	134	190.67	303.67	413.25	252.67
Calcium (mg/l)	G I (CON)	20.75	23	23.5	18.33	35.25	22	78.5	38.67	107.67	45.33	95.25	96
	G II (DE)	22.5	18.75	35	24.3	30	47	58	69.33	85	80	103.5	96.67
	G III (DH)	21.5	33	33.5	42.67	34.25	40.5	51.75	82.25	55,5	82.25	100.75	128.25
Inorganic phosphorous (mg/l)	G I (CON)	19.85	23.6	15.37	29.65	36.25	58.8	65.77	54.95	76.47	64.87	83.32	49.3
	G II (DE)	17.17	18.62	29.95	14.6	26.4	31.1	34.6	41.97	40.93	52.17	47.5	79.75
	G III (DH)	39.73	18.77	20.37	74.77	25.95	40.4	47.63	61.47	60.1	65.57	60.37	121.95

GI (CON): Group 1 Control

G II (DE): Group 2 Dental Extraction

G III (DH): Group 3 Drill Hole

3.2. Radiological Data

3.2.1. In the Females

Female rats in the dental extraction group (GII) showed a pronounced pattern of initial decrease in dental BMD at week

2 (583 g/cm³) followed by significant recovery by week 6 (1477.33 g/cm³). This pattern reflects the bone remodeling process following extraction, with initial resorption followed by robust formation and mineralization.

Table 2. Mean values of radiological parameters studied during experimentation on female rats.

X-ray parameters	Experimental Groups	Time (in weeks)			P Value
		Week 2	Week 4	Week 6	
Dental BMD (g/cm ²)	GI (CON)	1195.5	1003	1396.67	/
	GII (DE)	583	959	1477.33	0.6577
	G III (DH)	1031.75	1302	1413	0.5988
Symphyseal BMD (g/cm ²)	G I (CON)	565	1128.75	1049.25	/
	G II (DE)	316.33	774.67	1745.25	0.9984
	G III (DH)	839.67	1166	1205.67	0.9903
Mandibular horizontal branch BMD (g/cm ²)	G I (CON)	487.25	892.67	825.67	/
	G II (DE)	693	787.67	1041.75	0.8927
	G III (DH)	1045.75	768.33	924.33	0.7698

GI (CON): Group 1 Control

G II (DE): Group 2 Dental Extraction

G III (DH): Group 3 Drill Hole

BMD: Bone Mineral Density

3.2.2. In the Males

Unlike females, male rats showed higher baseline BMD values and distinct patterns of recovery. The symphyseal BMD in the drill hole group (GIII) demonstrated a remarkable

increase from 745.25 g/cm² at week 2 to 1598.25 g/cm² at week 6, representing a 114.5% increase and suggesting robust healing capacity in this region.

Table 3. Mean values of radiological parameters studied during the experiment in male rats.

X-ray parameters	Experimental Groups	Time (in weeks)			P Value
		Week 2	Week 4	Week 6	
Dental BMD (g/cm ²)	GI (CON)	729	944.5	1036.25	/
	GII (DE)	984	1194	1152.75	0.2315
	G III (DH)	1114.25	1374	1168.25	0.0674
Symphyseal BMD (g/cm ²)	G I (CON)	346.75	866	1326.33	/
	G II (DE)	556.5	759.5	1120.75	0.9922
	G III (DH)	745.25	1150.5	1598.25	0.8607
Mandibular horizontal branch	G I (CON)	355.33	731.25	773.75	/

X-ray parameters	Experimental Groups	Time (in weeks)			
		Week 2	Week 4	Week 6	P Value
BMD (g/cm ²)	G II (DE)	617	766.5	971.33	0.7155
	G III (DH)	627.5	1075.75	833	0.5882

GI (CON): Group 1 Control

G II (DE): Group 2 Dental Extraction

G III (DH): Group 3 Drill Hole

BMD: Bone Mineral Density

3.3. Linear Correlation Study in Group II

3.3.1. In the Females

The perfect positive correlation ($r=1$) between alkaline phosphatase and dental BMD at week 4 suggests that this enzyme could serve as an excellent predictor of bone formation activity at this critical timepoint. The perfect negative correlation with phosphorus ($r=-1$) at the same week may reflect active mineral utilization during peak bone matrix production.

Table 4. Pearson coefficient (r) between dental bone mineral density and biochemical parameters in Group II females.

Biochemical parameters / Dental BMD	Time (in weeks)		
	S2 r	S4 R	S6 r
Alkaline phosphatase / Dental BMD	0.7	1	0.7
Phosphorus / Dental BMD	-0.1	-1	-0.3
Calcium / Dental BMD	0.2	0.4	0.5

3.3.2. In the Males

In males, calcium demonstrated the strongest correlation with dental BMD ($r=0.9$ at week 4), suggesting potential sex-based differences in biomarker patterns during bone healing. Week 4 again emerged as a pivotal timepoint for marker-BMD correlations in the dental extraction model.

Table 5. Pearson coefficient (r) between dental bone mineral density and biochemical parameters in Group II males.

Biochemical parameters / Dental BMD	Time (in weeks)		
	S2 r	S4 r	S6 r
Alkaline phosphatase / Dental BMD	0.2	0.8	0.6

Biochemical parameters / Dental BMD	Time (in weeks)		
	S2 r	S4 r	S6 r
Phosphorus / Dental BMD	-0.2	0	0.2
Calcium / Dental BMD	0.4	0.9	0.6

3.4. Linear Correlation Studies in Group III

3.4.1. In the Females

In the drill hole model (Group III), calcium showed the strongest correlation with symphyseal BMD in females, reaching $r=0.8$ at week 4. This suggests that serum calcium levels may be particularly informative for monitoring healing in this type of mandibular defect.

Table 6. Pearson coefficient (r) between symphyseal bone mineral density and biochemical parameters in Group III females.

Biochemical parameters / Symphyseal BMD	Time (in weeks)		
	S2 r	S4 r	S6 r
Alkaline phosphatase / Symphyseal BMD	0.6	0.7	0.6
Phosphorus / Symphyseal BMD	0.2	-0.7	-0.4
Calcium / Symphyseal BMD	0.6	0.8	0.7

3.4.2. In the Males

The shift in phosphorus correlation from positive during early healing (weeks 2-4) to negative during late healing (week 6) suggests dynamic changes in mineral metabolism throughout the bone repair process. This pattern may reflect initial mineral recruitment followed by later remodeling and maturation phases.

Table 7. Pearson coefficient (*r*) between symphyseal bone mineral density and biochemical parameters in Group III males.

Biochemical parameters / Symphyseal BMD	Time (in weeks)		
	S2 <i>r</i>	S4 <i>r</i>	S6 <i>r</i>
Alkaline phosphatase / Symphyseal BMD	0.6	0.8	0.7
Phosphorus / Symphyseal BMD	0.5	0.3	-0.6
Calcium / Symphyseal BMD	0.7	0.8	0.7

4. Discussion

4.1. Linear Correlation Study in Group II Males and Females

Our results reveal a very strong positive linear correlation during week 4 between alkaline phosphatase (ALP) and dental bone mineral density (BMD) in Group II females, reaching a perfect correlation ($r = 1$). This suggests that in the females, ALP is a reliable indicator of bone remodeling activity and mineralization during the dental bone consolidation process. This finding aligns with recent studies by Zheng et al. (2021), who demonstrated similar correlations between biochemical markers and mandibular healing parameters [9].

Similarly, we observed a very strong positive linear correlation between calcium and dental BMD in the males during week 4 ($r = 0.9$). This result underlines the importance of calcium in the bone mineralization process in males, particularly during the remodeling phase [8]. A strong positive linear correlation during week 4 was also observed between ALP and dental BMD in males ($r = 0.8$).

These findings suggest that ALP and calcium, particularly during week 4 of our experiment, are relevant markers for assessing mandibular bone consolidation. Their measurement could potentially allow for monitoring healing progress and detecting any delays in consolidation, thus offering a complementary approach to CT scanning. Our results also indicate that Week 4 represents a critical phase in bone remodeling, as evidenced by the strong correlations observed between biochemical markers and BMD. This observation aligns with the known timeline of bone healing, where the transition from the reparative to the remodeling phase typically occurs around this period [1, 3]. This observation may be linked to the continued growth of the extracted dental incisor, which actively mobilizes the elements required for bone and tooth regeneration [3, 9].

4.2. Linear Correlation Study in Group III Males and Females

In Group III (drill hole group), strong positive linear correlations were observed between calcium and ALP, and

symphyseal BMD at week 4 of experimentation. This observation reinforces the notion that ALP and calcium are important markers of bone remodeling, as previously reported [3, 8]. Their correlation with symphyseal BMD suggests that they are also relevant for assessing bone consolidation in this region.

The differentiated patterns of correlation between phosphorus and BMD (varying from positive to negative correlations at different time points) suggest a complex and dynamic role of phosphorus in the bone healing process. Initially, phosphorus may contribute to bone mineralization, but its role may change during later stages of healing, possibly reflecting shifts in mineral deposition and resorption processes [9, 10].

Gender differences in correlation patterns indicate potential hormonal influences on bone metabolism and healing processes, which merit further investigation in future studies with larger sample sizes.

5. Conclusion

Biochemical markers such as alkaline phosphatase and calcium, as well as bone mineral density (BMD), are proving to be invaluable tools for monitoring bone healing. Their ease of use and ability to reflect the activity of phosphocalcium metabolism at each phase of healing make them relevant indicators for assessing the efficacy of osteo-inductive or osteo-conductive treatments.

Our study demonstrated strong correlations between these markers and bone mineral density at critical time points during healing, with distinct patterns observed between males and females and between different types of bone lesions. During Week 4 emerged as a particularly important time point for evaluation, showing the strongest correlations between biochemical markers and BMD, which could guide the timing of clinical assessments in future applications. The complementary nature of biochemical marker analysis and BMD measurements provides a more comprehensive approach to monitoring bone healing than either method alone. The strong positive correlations between alkaline phosphatase, calcium, and BMD support their use as a panel of indicators for assessing healing progression.

In the future, these markers could be integrated into clinical protocols to optimize the management of patients with fractures, delayed consolidation, or bone defects, enabling objective assessment of healing progression and personalized adaptation of therapeutic interventions. Longitudinal studies in human populations would be valuable to validate these findings and establish reference ranges for clinical application. Additionally, exploring the role of cellular components, such as mesenchymal stem cells and their interaction with immune factors [11], could provide deeper insights into the molecular mechanisms underlying the correlations observed between biochemical markers and bone mineral density.

Abbreviations

ALP	Alkaline Phosphatase
BMD	Bone Mineral Density
CON	Control (Group I)
DE	Dental Extraction (Group II)
DH	Drill Hole (Group III)
GI	Group I (Control)
GII	Group II (Dental Extraction)
GIII	Group III (Drill Hole)
UI/L	International Units per Liter
W0-W6	Week 0 to Week 6
S2/S4/S6	Scan at Week 2/Week 4/Week 6
r	Pearson's Correlation Coefficient
CT	Computed Tomography
DMO	Densité Minérale Osseuse (French Equivalent of BMD)
3R	Replacement, Reduction, Refinement (Ethical Principles for Animal Experimentation)

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Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

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