



The Impacts of Teriparatide Treatment on Bone Alkaline Phosphatase, Calcium and Phosphate Serum Levels in Rats

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ABSTRACT

Trauma, tooth loss, cancer surgery, congenital malformations, periodontal disease, and oral-maxillofacial surgery are all major causes of bone loss, deformity, or fracture. Bone healing is a physiologically complicated process that also incorporates mechanical factors. Too many doubts concerning bone regeneration and the conditions that promote it have persisted for far too long. To assess the impact of systemic teriparatide therapy on mandibular bone defects healing. Forty male albino rats were randomly divided into two groups: the control group and the treatment group, with 20 rats per group. All rats had the same surgical technique. A small hole was created in the mandible, measuring 3 mm in diameter and 3 mm in depth, and then left unoccupied. The treatment group received a daily subcutaneous injection of Teriparatide at a dose of 10 µg/kg. Animals were subjected to euthanasia at four distinct time intervals (7, 14, 21, and 28) days. Biochemical analyses were done. Teriparatide was shown to elevate serum levels of bone alkaline phosphatase (BALP), elevate serum calcium concentration levels, and lower serum phosphate. There were statistically significant differences in the levels of BALP between the control group and the treated group at 7, 14, and 21 days after the surgical procedure and there was a statistically significant difference in serum calcium levels between the treated group and the control group on day 14 of the experiment. The findings of this research suggest that the systemic administration of teriparatide resulted in an acceleration of bone healing. This was evidenced by a notable elevation in bone alkaline phosphatase levels as seen in the biochemical analysis. Teriparatide slightly elevated serum calcium levels and lowered serum phosphate levels because it is a synthetic parathyroid hormone.

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INTRODUCTION

Bone is a kind of calcified living connective tissue that is metabolically active and makes up the bulk of the skeleton (Wawrzyniak and Balawender, 2022). Bone is a part of the human body that serves to shape the body, supports muscle, shields essential organs, and forms hematopoietic cells. It is often regarded as one of the toughest materials generated by any biological process. Bone has the ideal properties of rigidity, flexibility, and lightweight since it is a composite material consisting of both mineral and collagen (Mohammed *et al.*, 2022).

Alkaline phosphatase (ALP) is a metalloenzyme that consists of various isoenzymes.

Every isoenzyme is a glycoprotein that is linked to the cell membrane. The expression of ALP is notably elevated in the cellular composition of mineralized tissue, where it serves an essential function in the process of hard tissue creation (Sharma *et al.*, 2014). Alkaline phosphatase (ALP) enhances the local rates of inorganic phosphate and promotes mineralization while concurrently decreasing the extracellular concentration of pyrophosphate, which acts as an inhibitor of mineral formation (Vimalraj, 2020). Bone alkaline phosphatase (BALP) is a remarkable marker of bone formation originating from osteoblasts and has a favourable correlation with the process of bone synthesis (Mahmood and Taqa, 2022; Naji *et al.*, 2022).

Teriparatide is an effective osteoanabolic agent that is a recombinant form of human parathyroid hormone (rhPTH). It contains the first 34 amino acids of human PTH, which are identical to the N-terminal portion of the hormone (Bhuyan *et al.*, 2017). The medicine received approval from the Food and Drug Administration (FDA) in November 2002 for the purpose of treating osteoporosis in those who are at a heightened risk of experiencing fractures, such as postmenopausal women and men with primary or hypogonadal osteoporosis (Yoshiga *et al.*, 2022). In contrast to antiresorptive drugs such as bisphosphonates or raloxifene, which exert their effects by impeding bone resorption, teriparatide seems to elicit an initial stimulation of osteoblast maturation and activity, leading to the generation of new bone tissue (Canalis, 2018). The study aimed to investigate the effect of systemic teriparatide administration on mandibular bone healing, bone alkaline phosphatase serum levels, and serum calcium and phosphate levels in experimental rats.

MATERIALS AND METHODS

Ethical approval

The research was approved by the Research Ethics Committee and Scientific Committee, Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Mosul, under approval number (UoM. Dent. 24/23), on February 27, 2023.

Animals

Forty male albino rats, ranging in age from 6-7 months and weighing between 300 and 350 grams, were employed in the investigation. The animal was housed in specialized cages at the University of Mosul, College of Dentistry's Animal House, where the temperature ranged between (22-24) °C. Under the watchful eye of a veterinarian, who checked on the animals before and after surgery, the animals were housed in regular circumstances and provided with standard meals and water. Animals were divided into 2 groups (control and treatment groups); each group consisted of 20 rats. The digital electronic scale was used to measure the rat's body weight. After that, a sedative analgesic solution (xylazine®) of 10 mg/kg and a general anesthetic agent (ketamine®) of 90 mg/kg were injected intraperitoneally in rapid succession to induce anaesthesia in the animal. Using an electric hair clipper and a 10% povidone iodine solution, the submandibular region of the animal was shaved while it was resting supine. Using scalpel blade number 15, a linear incision of 15 millimeters in length was performed on the skin, running parallel to the inferior edge of the mandible. Soft tissue was dissected away, the periosteum was lifted using a

Howarth periosteal elevator, and the mandibular bone was exposed. Next, a hole (3mm in diameter and 3 mm in depth) was drilled 5 mm posterior to the last molar tooth using a portable dental engine with a straight handpiece and a rounded carbide bur at a slow speed of 2000 rpm while irrigating vigorously with distilled water. The wound was cleaned, sutured, and disinfected.

Post-Surgical Procedure

Animal Care

Expert veterinarians monitored the rats' food (soft standardized diet), and physical activity after surgery, and the animals were kept in separate cages throughout the anesthetic recovery period. Following surgery, all rats quickly resumed their normal behavior and diet (within 3 to 5 hours).

Experimental substances

Injectable Teriparatide (Forsteo) was imported from Turkey and administered to the rats in the treatment group 1st day after the surgical procedure. Every day, all except the control group rats got a subcutaneous injection of 10 µg/kg teriparatide for 28 days. Fig. (1).



Fig. 1: Teriparatide injection.

Serum collection

The blood was collected from each rat on the sacrificing date of the experimental procedure (7, 14, 21 and 28 days). After anaesthetizing the rats using light ether anaesthesia for a few seconds, sterile microhematocrit capillary tubes were used to collect the blood from a retro-orbital venous plexus into a sterile gel tube without anticoagulant. The blood samples were incubated at room temperature for 30 minutes, separating the serum by using a centrifuge at 3000 rpm for 10 minutes. The serum was carefully transferred using a micropipette into an Eppendorf tube and thereafter stored in a freezer at a temperature of -20°C. This refrigeration was done in order to facilitate the thawing process since the serum is designed for examination via a microplate enzyme-linked immunosorbent assay (ELISA) reader for Rat Bone alkaline phosphatase (BALP) from MyBiosource and for serum calcium and phosphate analysis.

Statistical Analysis

The data were expressed as mean ± S.D., and then statistically analyzed by independent sample T-test was used to examine the differences between the control and the treated groups. A one-way analysis of variance (ANOVA) with a post-hoc Duncan's test was used to examine the differences within group in different intervals. The level of significance was at $p \leq 0.05$.

RESULTS

Bone Alkaline Phosphatase

On day 7:

The independent sample T test showed a significant difference between the control group (0.47 ± 0.01) in comparison with the treated group (0.78 ± 0.05) at the 7-day postoperative period, as shown in figure (2) and table (1).

Table 1: BALP concentration (ng/ml) between the control and the teriparatide groups on (7, 14, 21 and 28 days) of the experiment.

Days	7 days	14 days	21 days	28 days
Control	0.47 ± 0.01 ^{Aa}	0.71 ± 0.10 ^{Ba}	0.85 ± 0.09 ^{Ca}	0.71 ± 0.08 ^{Ba}
Treated	0.78 ± 0.05 ^{Ab}	1.08 ± 0.10 ^{Bb}	1.22 ± 0.21 ^{Bb}	0.78 ± 0.02 ^{Aa}

Data were described as (Mean + SD). Different capital letters mean there are significant differences in the same row. Different small letters mean there are significant differences in the same column.

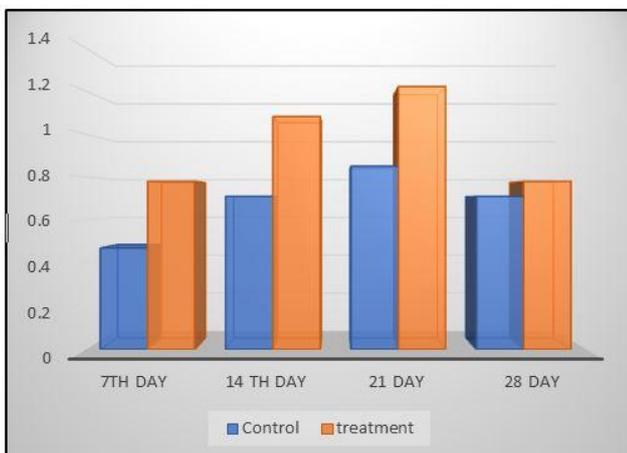


Fig. 2 : BALP concentration in experimental rats (ng/ml) between the control and teriparatide groups of the study on (7, 14, 21 and 28) postoperative days (t-test for 20 animals\ group).

On day 14:

There was a significant difference between the control group (0.71 ± 0.10) and the treated group (1.08 ± 0.10) at the 14-day postoperative period. as shown in figure (2) and table (1).

On day 21:

There was a significant difference between the control group (0.85 ± 0.09) and the treated group (1.22 ± 0.21) at the 21-day postoperative period. as shown in figure (2) and table (1).

On day 28:

There was no significant difference between the control group (0.71 ± 0.08) and the treated group (0.78 ± 0.02) at the 28-day postoperative period, as shown in figure (2) and table (1).

Serum calcium levels

On Day 7:

The serum calcium levels in the treated group were slightly elevated compared to the control group. There was no significant difference between the control group (9.04 ± 0.04) and the treated group (9.50 ± 0.32) on day 7 of the experiment, as shown in Fig.3 and table (2).

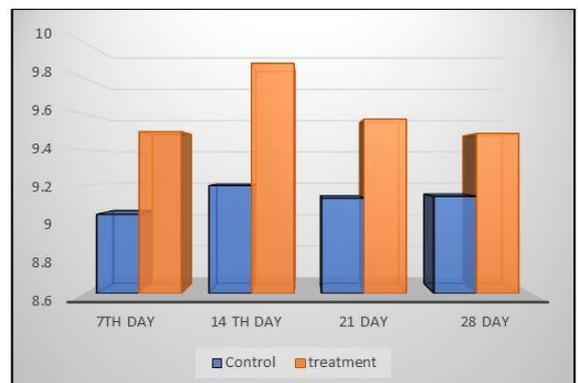


Fig. 3: Calcium Levels mg/dl between the control group and the teriparatide group at 7, 14, 21 and 28 days after the surgical procedure (t-test for 20 animals\ group).

Table 2: Calcium Levels mg/dl between the control group and the teriparatide group at 7, 14, 21 and 28 days of the experiment.

Days	7 days	14 days	21 days	28 days
Control	9.04 ± 0.04 ^{Aa}	9.2 ± 0.08 ^{Aa}	9.13 ± 0.16 ^{Aa}	9.14 ± 0.04 ^{Aa}
Treated	9.50 ± 0.32 ^{Aa}	9.88 ± 0.08 ^{Ab}	9.57 ± 0.49 ^{Aa}	9.49 ± 0.24 ^{Aa}

On Day 14:

There was a significant difference between the control group (9.2 ± 0.08) and the treated group (9.88 ± 0.08) at the 14-day postoperative period, as shown in Fig.3 and table (2).

On Day 21:

There was no significant difference between the control group (9.13 ± 0.16) and the treated group (9.57 ± 0.49) at the 21-day postoperative period, as shown in Fig.3 and table (2).

On Day 28:

There was no significant difference between the control group (9.14 ± 0.04) and the treated group (9.49 ± 0.24) at the 28-day postoperative period, as shown in Fig.3 and table (2).

Serum phosphate levels

On Day7:

There was no significant difference between the control group (7.1 ± 0.62) and the treated group (6.46 ± 0.47) on day 7 of the experiment as shown in Fig.4 and table (3).

On Day14:

There was no significant difference between the control group (6.56 ± 0.41) and the treated group (6.0 ± 0.06) on day 14 of the experiment, as shown in Fig.4 and table (3).

On Day 21:

There was no significant difference between the control group (6.03 ± 0.75) and the treated group (5.63 ± 0.15) on day 21 of the experiment, as shown in Fig.4 and table (3).

On Day 28:

There was no significant difference between the control group (7.3 ± 0.52) and the treated group (6.56 ± 0.58) on day 28 of the experiment, as shown in Fig.4 and table (3).



Fig 4: Phosphate Levels mg/dl between the control group and the teriparatide group at 7, 14,21 and 28 days after the surgical procedure (t-test for 20 animals\ group).

Table 3: Phosphate Levels mg/dl between the control group and the teriparatide group at 7, 14, 21and 28 days of the experiment.

Days	7 days	14 days	21 days	28 days
Control	7.1 ± 0.62 ^{ABCa}	6.56 ± 0.41 ^{ABCa}	6.03 ± 0.75 ^{ABa}	7.3 ± 0.52 ^{ACa}
Treated	6.46 ± 0.47 ^{ABCa}	6.0 ± 0.06 ^{ABCa}	5.63 ± 0.15 ^{ABa}	6.56 ± 0.58 ^{ACa}

DISCUSSION

The use of diverse bone substitutes is crucial in serving as alternative graft materials to achieve effective bone restoration (Taqa et al., 2023). Teriparatide (TP) has gained significant traction in the management of osteoporosis in recent years, mostly owing to its capacity to enhance bone production via its anabolic properties. Furthermore, TP has potential use in the repair of bone defects (Keskinrüzgar et al., 2015). The effects of continuous administration of parathyroid hormone (PTH) compared to intermittent administration vary in terms of their impact on osteogenesis and osteoclastic activity. Continuous administration leads to a net loss of bone due to increased bone resorption, while intermittent administration promotes a net gain in bone via increased bone deposition (Zandi et al., 2019).

In this study, the teriparatide-treated group exceeded the control group in serum bone alkaline phosphatase concentration levels, and there were statistically significant differences between the teriparatide group and the control group at (7, 14, 21 days) postoperative periods of the experiment unlike the 28th postoperative period, where there was no significant difference between the experimental groups. These findings correlate with the histological findings of our experiment, which showed that teriparatide enhanced and accelerated mandibular bone defect healing in the treated group at the different treatment intervals. In addition, the study revealed that there were transient elevations in serum calcium levels in the treated group in comparison with the control group, with a statistically significant difference at day 14 after the surgical procedure, and slight decreases in serum phosphate levels in the treated group in comparison with the control group at all the treatment intervals.

A biomarker is a measurable indicator of a biological activity, pathogenic process, or pharmacological response to a treatment intervention (Colloca *et al.*, 2020). It was found that elevated serum alkaline phosphatase activity was associated with elevated osteoblastic activity in bone. This link provides evidence to support the interpretation of serum alkaline phosphatase activity in the context of bone disorders. Plasma membrane-bound glycoprotein alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate monoesters, leading to the production of inorganic phosphate (Sharma *et al.*, 2014). The enzyme serum alkaline phosphatase has an isoform that is found only in bones called bone alkaline phosphates (BALP). It is a glycoprotein produced by osteoblasts and serves as an indicator of the cellular activity of these cells in bone metabolism (Bonnick *et al.*, 2013). BALP is a remarkable marker of bone formation produced by osteoblasts and has a favourable link with the rate of bone synthesis (Hussein and Taqa, 2021).

Both teriparatide and original parathyroid hormone (PTH) elicit their physiological effects by specifically attaching to cell-surface receptors with high affinity, which are situated on osteoblasts and tubular cells of the kidneys. Both substances exhibit similar binding affinity to the receptors and elicit identical physiological responses in relation to bone and kidney functions (Le Henaff and Partridge, 2020). Teriparatide induces osteoblastic activity and promotes the production of new bone tissue. Elevations in levels of formation markers of bone occur faster than elevations in resorption markers of bone, establishing the 'anabolic window'. There may be a correlation between the first alterations in biochemical markers associated with bone formation and subsequent enhancements in bone structure and bone mineral density (BMD) (Blick *et al.*, 2008).

The observed augmentation in bone formation subsequent to teriparatide treatment is likely attributable to the stimulation of circulating osteoblast precursors, prompting their maturation, as well as the differentiation of lining osteoblasts. These processes result in an elevated osteoblast count, prevention of osteoblast cell death, heightened apposition rate of minerals, increased osteoblast activity, and enhancement of bone microarchitecture (Zandi *et al.*, 2019).

Teriparatide has similar effects to the natural parathyroid hormone (PTH) on the regulation of calcium and phosphate levels in the body. Specifically, it leads to an elevation in blood calcium levels and a reduction in serum phosphate levels, hence influencing calcium and phosphate balance (Sarma *et al.*, 2021). The well-known effects of PTH

on kidneys and bones are responsible for these outcomes. Parathyroid hormone (PTH) has the ability to enhance the reabsorption of calcium in the distal tubules of the kidney. Additionally, it inhibits the reabsorption of phosphate in the proximal tubules. Moreover, PTH activates the enzyme 1-alpha-hydroxylase in the proximal tubules, leading to the conversion of filtered 25-hydroxyvitamin D to 1,25-dihydroxy vitamin D, which is the most biologically active form of vitamin D. In the skeletal system, it releases calcium from the bone matrix into the circulatory system (Young *et al.*, 2022).

There were a number of limitations to this study. Primarily, the experiment was conducted on an animal model, which has a different physiology, drug metabolism, bone turnover, and likely bone repair process than humans. Second, no comparison was made between the effects of different dosages and durations of teriparatide treatment on bone healing; only the effects of a single dose (10 µg/kg/day) were studied. Third, the effects of teriparatide on bone healing over a longer period (more than 2 months) were not studied. Research into the long-term benefits of teriparatide treatment on bone defect repair is strongly encouraged, as are more investigations employing big animals and clinical trial research.

CONCLUSION

The present investigation examined the efficacy of administering teriparatide through daily subcutaneous injections in promoting bone healing responses. The findings revealed that teriparatide accelerated the bone healing process and increased the levels of bone alkaline phosphatase in the bloodstream, which serves as an indicator of bone formation. Additionally, the administration of teriparatide resulted in elevated serum calcium levels and decreased serum phosphate concentrations, consistent with the expected effects of parathyroid hormone.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the research data and tools used in this study.

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